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Pharmacological sensitivity and gene expression analysis of the tibial nerve injury model of neuropathic pain

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Abstract

The tibial nerve injury model is a novel, surgically uncomplicated, rat model of neuropathic pain based on a unilateral transection (neurotomy) of the tibial branch of the sciatic nerve. The aim of the present study was to describe some behavioral and molecular features of the model, and to test its sensitivity to a number of drugs which are currently used for the treatment of neuropathic pain. The model was characterized by a pronounced mechanical allodynia which was present in all subjects and a less robust thermal hyperalgesia. Mechanical allodynia developed within 2 weeks post-surgery and was reliably present for at least 9 weeks. Neurotomized rats showed no autotomy and their body weight developed normally. Gene expression in ipsilateral L5 dorsal root ganglia, analyzed by quantitative polymerase chain reaction (PCR), showed a pronounced up-regulation of galanin and vasointestinal peptide (VIP). This up-regulation developed rapidly (within 1 to 2 days following neurotomy) and remained present for at least 12 days. On the other hand, expression of calcitonin gene-related peptide (CGRP) and substance P mRNA was down-regulated 12 days following neurotomy. Mechanical allodynia was completely reversed by morphine [minimal effective dose (MED): 8 mg/kg, i.p.] and partially reversed by carbamazepine (MED: 64 mg/kg, i.p.), baclofen (MED: 3 mg/kg, i.p.) and amitriptyline (trend for efficacy at 32 mg/kg, i.p.), but not by gabapentin (50–100 mg/kg, i.p.). The finding that the tibial nerve injury model shows a robust and persistent mechanical allodynia which is sensitive to a number of established analgesics, as well as a gene expression profile which is compatible with that obtained in other models of neuropathic pain, further supports its validity as a reliable and surgically uncomplicated model for the study of neuropathic pain.

Keywords: Analgesic; Animal model; Nociception; Gene expression; Polymerase chain reaction (PCR); Quantitative PCR; (Rat)

1. Introduction

Peripheral nerve injury often results in chronic neuropathic pain, characterized by hyperalgesia and allodynia to mechanical (tactile) and thermal (heat or cold) stimuli, as well as spontaneous pain (Koltzenburg, 1998). Thus far, most animal models of chronic neuropathic pain have been based on a unilateral (partial) ligation of the sciatic or spinal nerves in the rat (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). Comparison of these models within and between different laboratories has indicated that they show differences in (a) the time-course and the degree of allodynia and hyperalgesia against mechanical and/or thermal stimuli, (b) the occurrence of spontaneous pain, autotomy and inflammatory processes, and (c) in the sensitivity to sympathectomy and pharmacotherapy (e.g., Choi et al., 1994; Desmeules et al., 1995; Kim et al., 1997; Martin and Eisenach, 2001). It can be assumed that part of this variation derives from differences in the degree of ligation and ligation materials, which result in differential spectra of fiber loss and inflammation (Basbaum et al., 1991; Maves et al., 1993), differences in rat strain (Yoon et al., 1999) and, at least for the spinal nerve ligation model, from intra-individual differences in rat sciatic nerve anatomy (Asato et al., 2000).

In an attempt to avoid the complexity of ligation methods, a number of models have recently been introduced which are based on a unilateral transection (neurotomy) of one or more of the three distal branches of the sciatic nerve (i.e., sural, tibial and/or common peroneal nerve). Thus, Lee et al. (2000) reported that the level of allodynia and spontaneous

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pain was differentially affected after different combinations of transection of these three distal branches. Decosterd and Woolf (2000) and Hofmann et al. (2000) have described models based on transection of the tibial and common peroneal nerves (spared nerve injury model), or single tibial nerve transection (tibial nerve injury model), respectively. Although the pharmacological sensitivity of the models based on nerve branch transection has not yet been studied extensively, Erichsen and Blackburn-Munro (2002) recently reported that the efficacy of a number of clinically used compounds in the spared nerve injury model differed from that obtained in other models, possibly reflecting mechanistically different consequences of the particular injury.

The aim of the present study was to describe some behavioral and molecular features of the tibial nerve injury model, and to test its sensitivity to a number of analgesic drugs with different mechanisms of action. The model may offer some advantages because of its relatively simple surgical procedure, avoiding the potential variability of ligation techniques, and because it most likely minimizes the contribution of inflammatory processes, due to the absence of ligation material (Lindenlaub and Sommer, 2000). Behavioral characterization focussed on the time-course of mechanical allodynia and thermal (heat) hyperalgesia. In addition, we determined whether a similar profile of gene regulation was obtained compared to other models of neuropathic pain, i.e., up-regulation of galanin and vasointestinal peptide (VIP) and down-regulation of calcitonin gene-related peptide (CGRP) and substance P (Bridges et al., 2001; Honore et al., 2000; Martin and Eisenach, 2001; Woolf and Salter, 2000). Pharmacological validation was performed with morphine, amitriptyline, carbamazepine, gabapentin and baclofen, compounds which are currently used for the treatment of neuropathic pain of different aetiology (Sindrup and Jensen, 1999).

2. Material and methods

2.1. Animals and housing conditions

Male Wistar rats (180–200 g; strain HsdCpb:WU) were housed in groups of six 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. Experimental protocols and conditions conform with the local regulations on animal welfare and with the Ethical Guidelines of the International Association for the Study of Pain (Zimmermann, 1983).

2.2. Surgery and tissue preparations

Rats were accustomed to the laboratory conditions for at least 3 days prior to surgical manipulations. The animals were randomly assigned to control, sham-operated or tibial nerve injury groups with $n = 7–8$ per group (gene expression and behavioral time-course study) or $n = 9–12$ per group (pharmacological studies). Tibial nerve injury was performed under pentobarbital anaesthesia (Nembutal, Sanofi, Libourne, France). Distal to the trifurcation of the left sciatic nerve, the tibial branch of the sciatic nerve was transected, whereas the sural and common peroneal nerves remained uninjured. Sham-operated animals were treated identically to tibial nerve injury animals, except for the tibial nerve transection. The control groups did not receive surgery. For the gene expression studies, rats were sacrificed 1, 2, 5 or 12 days after surgery. L5 dorsal root ganglia from single animals were harvested and snap frozen with liquid nitrogen. All subsequent analyzes were performed with non-pooled samples.

2.3. Behavioral and pharmacological studies

Prior to behavioral testing, the animals were accustomed to the test cages for 5 min. Assessment of the development and maintenance of mechanical allodynia and thermal hyperalgesia was performed in a tibial nerve injury and a sham-operated control group, at different time points following surgery (i.e., day 13, 19, 26, 33, 41, 58, 64 and 67 post-surgery). Mechanical allodynia was measured by means of a pressure transducer (electronic von Frey Anesthesiometer, IITC-Life Science Instruments, Woodland Hills, CA). In short, the animals were placed in a test cage with a wire mesh floor, and the tip of the anesthesiometer was applied to the middle of the plantar surface of the operated hindpaw. Withdrawal threshold was measured once per trial and expressed as tolerance level in grams (g). Thermal hypersensitivity of the operated hind limb was measured using the plantar test (Ugo Basile, Comerio, Italy) as described by Hargreaves et al. (1988). In short, 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. Pain sensitivity was recorded once per trial as withdrawal latency in seconds with a cut-off time of 32 s.

Pharmacological testing occurred in different collectives of tibial nerve injury and sham-operated groups, and was restricted to the assessment of mechanical allodynia. Baseline allodynia was checked on the day before pharmacological testing in order to ascertain behavioral pathology. The effect of acute i.p. administration of amitriptyline (16 and 32 mg/kg, $t = 60$ min; 2 weeks post-surgery), carbamazepine (32 and 64 mg/kg, $t = 30$ min; 3 weeks post-surgery), baclofen (1 and 3 mg/kg, $t = 60$ min; 3 weeks post-surgery), gabapentin (50 and 100 mg/kg, $t = 60$ min; 6 weeks post-surgery) and morphine (4 and 8 mg/kg, $t = 30$ min; 7 weeks post-surgery) was tested after tibial nerve injury, and compared with the effect of i.p. administration of the respective vehicle in both tibial nerve injury and sham-surgery control rats.

2.4. Drugs

Morphine hydrochloride (Merck, Darmstadt, Germany), gabapentin (content of capsules of Neurontin®, Parke-Davis, Freiburg, Germany, extracted by the Chemistry Department of Bayer, Wuppertal, Germany), amitriptyline (RBI, Natick, MA), carbamazepine and baclofen (Sigma-Aldrich, Steinheim, Germany) were administered i.p. in an application volume of 2 ml/kg body weight. Morphine and gabapentin were dissolved in saline (0.9% NaCl). Amitriptyline was suspended in a solvent containing 1% dimethylsulfoxide (DMSO; Merck) and saline; carbamazepine in a solvent containing 2% DMSO, 10% cremophor (Cremophor EL®, Fluka Chemie, Buchs, Switzerland) and saline; and baclofen in a solvent containing 10% Tween 80 (Sigma-Aldrich) and saline.

2.5. RNA preparation and cDNA synthesis

For preparation of total RNA from dorsal root ganglia tissue samples, the method of Chomczynski and Sacchi (1987) was used. In short, RNA was isolated from the supernatant by incubation with RNAmatrix beads (Qbiogene, Heidelberg, Germany). After washing with RNA-washing solution (Qbiogene) and drying, the RNA was eluted from RNAmatrix beads at 55 °C with DEPC-H_2O (Diethylpyrocarbonate; Sigma, Deisenhofen, Germany). Approximately 1 μg of total RNA was reverse-transcribed into cDNA using Superscript II reverse transcriptase (Invitrogen, Karlsruhe, Germany) in 40-μl reaction mixture. cDNA synthesis and polymerase chain reaction (PCR) were performed as described previously (Siegling et al., 1994).

2.6. Quantitative PCR

Gene expression was quantified using the 7700 Sequence Detector (Taqman™) and the SYBR Green PCR Core Reagent-Kit, as described in the manufacturer's manual (Applied Biosystems, Foster City, CA). For preparation of a standard DNA, the gene of interest was amplified by PCR, separated by gel electrophoresis and isolated from the gel fragment (Qiaex II, Qiagen, Hilden, Germany). The isolated PCR product was diluted in several magnitudes and used as a standard for the real time PCR reaction in order to relate threshold cycle to template copy number. In a first PCR reaction the cyclophilin content of each cDNA sample was quantified. Cyclophilin served as an intrinsic control for variations in cDNA amounts. Before the quantification of genes of interest, all cDNAs were diluted so that their cyclophilin content did not vary by more than a factor of 10. Amplification of the standard curve was performed in triplicates; every cDNA sample in duplicates to control for tube internal failures. The values were averaged for each reaction. Data were normalized by referring mean values of the target gene to mean values of the housekeeping gene cyclophilin. The specificity of the PCR products was verified by gel electrophoresis (data not shown). PCR reactions were performed in 25-μl volumes with a final concentration of 300 nmol for each primer, with 95 °C for 30 s and 60 °C for 60 s, for 40 cycles. Primers for rat cyclophilin were designed as reported by Costigan et al. (1998). The other primers were designed according to exon 6 of the rat calcitonin gene, specific for calcitonin gene-related product (CGRP, accession number L00111; sense 5′-ACT GAA ACC CTT CTC CCT ATG and antisense 5′-ATG GTT CCA TTG AGT CAC AAC; yielding a 259-bp product), the rat galanin precursor mRNA sequence (accession number J03624; sense 5′-TCT GGG GCT CGG GAT GC and antisense 5′-GCT TGA GGA GTT GGC GGA AGA; yielding a 397-bp product), the rat substance P precursor (accession number X56306; sense 5′-GCC CAC AAG AGA ATG AGG ACA and antisense 5′-AGG AGA GCC AGG ACC CAG AT; yielding a 305-bp product), and the rat mRNA for VIP precursor (accession number X02341; sense 5′-GCC GTG TTA CAT AAA GC GC and antisense 5′-GCC AAT AAG ACA AAA TAC TGT; yielding a 319-bp product).

2.7. Statistical analysis

Behavioral and pharmacological data were analyzed by one-way analysis of variance (ANOVA), followed, where appropriate, by Tukey's post hoc comparisons. Because the gene expression data were not normally distributed, statistical analysis was performed by means of a Kruskal–Wallis one-way ANOVA, followed, where appropriate, by Mann–Whitney U tests. Effects were considered to be statistically significant if $P < 0.05$.

3. Results

3.1. Mechanical allodynia and thermal hyperalgesia

In an initial series of experiments, the heat sensitivities (plantar test) of different groups of rats after unilateral transection of single or multiple branches of the sciatic nerve (i.e., the tibial, sural and common peroneal nerve) were compared. In these preliminary experiments, it was found that transection of the tibial nerve (tibial nerve injury) and combined transection of the tibial and the common peroneal nerve resulted in a similar reduction of paw withdrawal latency (data not shown). In contrast, combined transection of the tibial and sural nerve did not change the withdrawal latency in the plantar test (data not shown). As tibial nerve injury resulted in the development of thermal hyperalgesia and the procedure consisted of minimal nerve injury in combination with only little tissue damage, it was decided to further characterize the time-course of thermal hyperalgesia as well as mechanical allodynia following this procedure.

Thermal hyperalgesia developed between 2 and 3 weeks following surgery, and lasted for about 6 weeks (Group

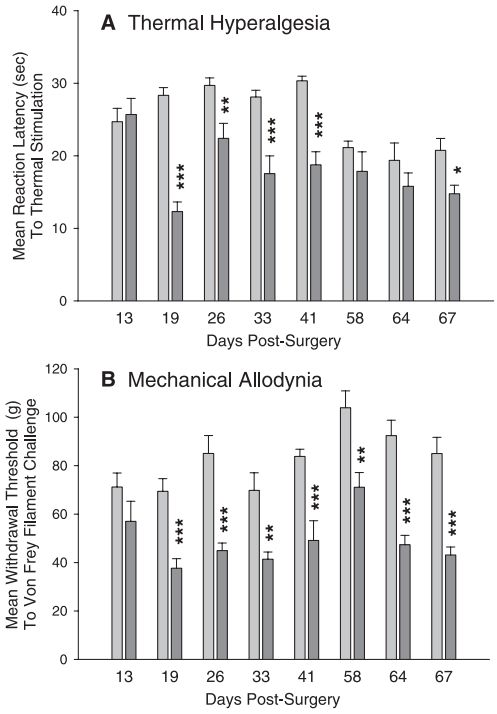

Fig. 1. Time-course of (A) thermal hyperalgesia and (B) mechanical allodynia after transection of the tibial branch of the sciatic nerve (tibial nerve injury; black bars) or sham-surgery (grey bars) in rats. Data were obtained from day 13 to day 67 after surgery, and show the mean (± S.E.M.) reaction time latency to thermal stimulation (expressed in seconds), or withdrawal threshold to electronic von Frey filament challenge (expressed in grams). *$P<0.05$, **$P<0.01$, ***$P<0.001$ versus sham-surgery group ($n=7-8$ per group).

effect: $F(1,12)=42.00$, $P<0.001$; Time effect: $F(7,84)=10.18$, $P<0.001$; Group × Time interaction effect: $F(7,84)=5.18$, $P<0.001$; with a statistically significant difference between the tibial nerve injury and sham-operated group at test day 19, 26, 33, 41 and 67 post-surgery; Fig. 1A). Within 2–3 weeks post-surgery, the tibial nerve injury animals also developed mechanical allodynia, which was more robust and of longer duration than the thermal hyperalgesia, as it lasted for at least 2 months (Group effect: $F(1,13)=81.10$, $P<0.001$; Time effect: $F(7,91)=6.64$, $P<0.001$; Group × Time interaction effect not significant: $F(7,91)=5.18$, $P=0.20$; with a statistically significant difference between the tibial nerve injury and sham-operated group at test day 19, 26, 33, 41, 58, 64 and 67 post-surgery; Fig. 1B). In order to avoid additional stress shortly after the surgical procedures, pain tests were not performed during the initial 2 weeks post-surgery.

3.2. General behavior

After tibial nerve injury, all animals held the affected paw in a raised posture with distinct pronation. Uplifting of the rats resulted in stretching of the toes of the unaffected limb (as observed in naive rats). However, the paw of the neurotomized hind limb remained closed. General behavior and social interactions were not obviously changed compared to naive and sham-treated rats, and autotomy of the operated limb did not occur. Body weight developed normally and identically in tibial nerve injury and sham-operated groups (data not shown).

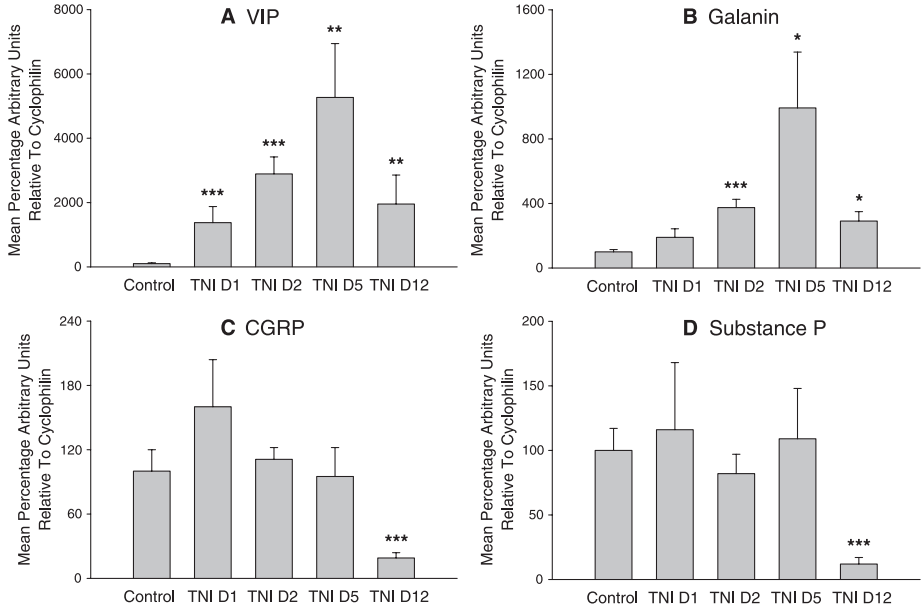

Fig. 2. Regulation of mRNA expression of (A) vasointestinal peptide (VIP), (B) galanin, (C) calcitonin gene-related peptide (CGRP) and (D) substance P in ipsilateral L5 dorsal root ganglia after transection of the tibial branch of the sciatic nerve (tibial nerve injury) of rats. Tissues were harvested from treatment-naive (Control), sham-operated and neurotomized (tibial nerve injury) animals ($n=7-8$ per group), at days (D) 1, 2, 5 and 12 post-surgery. Gene expression of VIP, galanin, CGRP, substance P and cyclophilin was quantified by real-time PCR. Values for mRNA expression are shown as the mean (± S.E.M.) of arbitrary units relative to cyclophilin mRNA expression. *$P<0.05$, **$P<0.01$, ***$P<0.001$ versus treatment-naive animals.

3.3. Gene expression analysis

mRNA expression of VIP was increased about 15- to 50-fold from day 1 to day 12 in ipsilateral dorsal root ganglia tissue after tibial nerve injury, as compared to treatment-naive control animals ($\chi^2_{(4)} = 21.01$, $P < 0.001$; with a statistically significant up-regulation at day 1, 2, 5 and 12 post-operation; Fig. 2A) and sham-surgery control animals (data not shown). Similarly, mRNA expression of galanin was increased about 3- to 10-fold from day 2 to day 12 in tibial nerve injury rats, as compared to treatment-naive control animals ($\chi^2_{(4)} = 14.97$, $P < 0.01$; with a statistically significant up-regulation at day 2, 5 and 12 post-operation; Fig. 2B) and sham-surgery control animals (data not shown). For both genes, the maximal increase of mRNA expression was obtained at day 5 post-surgery.

In contrast to VIP and galanin, expression of CGRP mRNA in ipsilateral dorsal root ganglia tissue was decreased after tibial nerve injury, as compared to treatment-naive control rats ($\chi^2_{(4)} = 19.12$, $P < 0.001$; with a statistically significant fivefold down-regulation at day 12 post-operation; Fig. 2C) and sham-surgery control rats (data not shown). Similarly, expression of substance P mRNA was decreased in tibial nerve injury rats, as compared to treatment-naive control rats ($\chi^2_{(4)} = 15.89$, $P < 0.01$; with a statistically significant eightfold down-regulation at day 12 post-operation; Fig. 2D) and sham-surgery control rats (data not shown).

3.4. Pharmacological analysis

Acute administration of morphine (8 and 16 mg/kg, i.p.) resulted in complete reversal of mechanical allodynia ($F(3,35) = 17.28$, $P < 0.001$; at 8 mg/kg, a statistically significant difference between vehicle-treated tibial nerve injury group and drug-treated tibial nerve injury group was obtained, and there was no longer a statistically significant difference between drug-treated tibial nerve injury group and vehicle-treated sham-operated group; Fig. 3A).

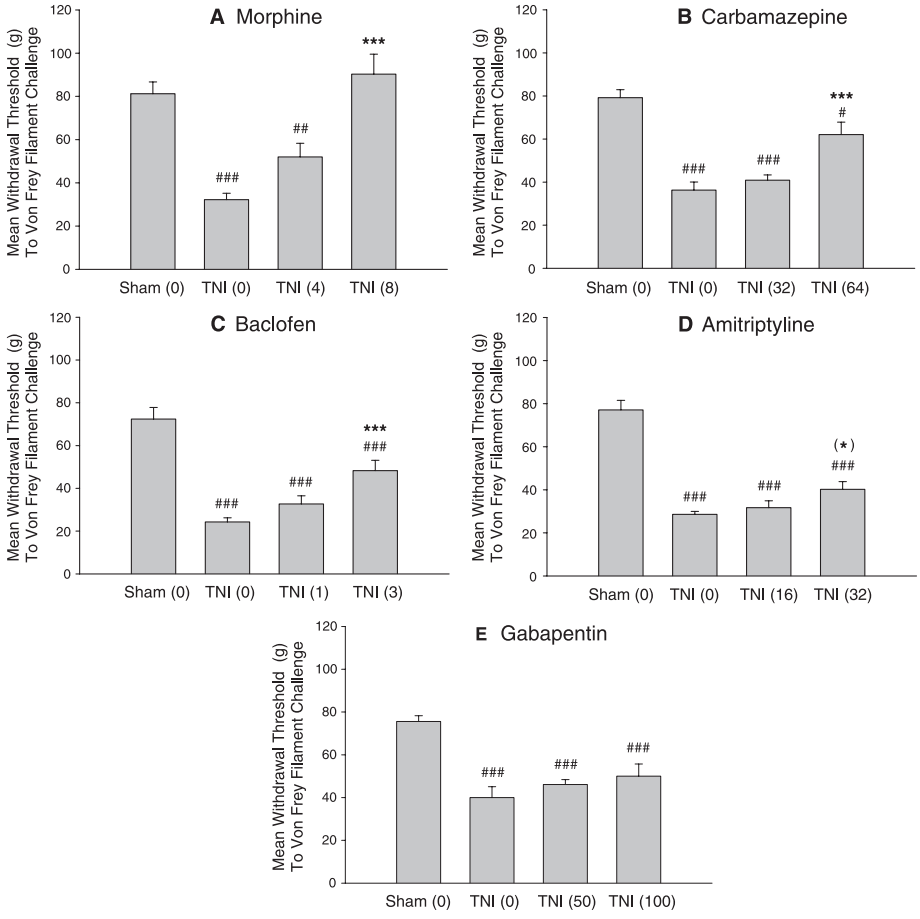

Fig. 3. Effect of acute i.p. administration of (A) morphine ($n = 9–12$ per group, $t = 30$ min), (B) carbamazepine ($n = 11–12$ per group, $t = 30$ min), (C) baclofen ($n = 11–12$ per group, $t = 60$ min), (D) amitriptyline ($n = 11–12$ per group; $t = 60$ min) and (E) gabapentin ($n = 11–12$ per group; $t = 60$ min) on mechanical allodynia after transection of the tibial branch of the sciatic nerve. Data show the mean (± S.E.M.) withdrawal threshold to electronic von Frey filament challenge, expressed in grams, in neurotomized (tibial nerve injury) or sham-surgery rats. Doses are expressed in mg/kg and are indicated in brackets. $^{(*)}P = 0.096$, $^{*}P < 0.05$, $^{***}P < 0.001$ versus vehicle-treated neurotomized (tibial nerve injury) group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ versus vehicle-treated sham-surgery group.

Carbamazepine (32 and 64 mg/kg, i.p.) and baclofen (1 and 3 mg/kg, i.p.) attenuated mechanical allodynia ($F(3,42)=22.47$, $P<0.001$ and $F(3,43)=26.19$, $P<0.001$; respectively; Fig. 3B and C). For both compounds, a statistically significant difference between the vehicle-treated tibial nerve injury group and the drug-treated tibial nerve injury group was obtained at the highest dose. However, the attenuation of mechanical allodynia was not complete, as there was still a statistically significant difference between the drug-treated tibial nerve injury group and the vehicle-treated sham-operated group.

Administration of amitriptyline (16 and 32 mg/kg, i.p.) resulted in a weak attenuation of mechanical allodynia ($F(3,42)=43.54$, $P<0.001$; the highest dose tended to increase the withdrawal threshold, but the effect fell short of statistical significance, $P=0.096$; Fig. 3D). Finally, gabapentin (50 and 100 mg/kg, i.p.) failed to affect mechanical allodynia (ANOVA indicated a Group effect: $F(3,42)=43.54$, $P<0.001$; but this effect was due to a statistically significant difference between the vehicle-treated sham-operated group and each tibial nerve injury group; Fig. 3E).

4. Discussion

A number of neuropathic pain models based on unilateral transection of one or more of the three distal branches of the sciatic nerve (i.e., sural, tibial and/or common peroneal nerve) have recently been introduced. Thus, Lee et al. (2000) reported that combined transection of the tibial and sural nerves (leaving the common peroneal nerve intact) produces robust symptoms of neuropathic pain. Decosterd and Woolf (2000) described a model based on transection of the tibial and common peroneal nerves (spared nerve injury model) and found a robust and prolonged occurrence of mechanical allodynia and hypersensitivity, as well as an increased thermal responsiveness. In the present study it was found that the tibial nerve injury model, a surgically uncomplicated neuropathic pain model based on a unilateral transection of the tibial branch of the sciatic nerve, showed a pronounced mechanical allodynia which coincided with a less robust thermal hyperalgesia. Comparison of the different models based on transection indicates that the profile of behavioral symptoms may be different depending on the particular transection. Thus, according to Lee et al. (2000), combined transection of the tibial and sural nerves results in a robust level of mechanical and cold allodynia, which is also found after tibial nerve injury, but which appears more pronounced as that obtained in the spared nerve injury model. The finding that mechanical allodynia was robust and long-lasting in the presently described tibial nerve injury model is consistent with the data reported by Lee et al. (2000). Although we offer additional evidence that tibial nerve injury also results in thermal (heat) hyperalgesia, it appears that this symptom is somewhat less robust than mechanical allodynia.

Previous studies indicate that transection of the tibial and common peroneus nerves results in a higher sensitivity towards sympathectomy than transection of the tibial and sural nerves (Decosterd et al., 2001; Lee et al., 2000). It remains to be determined to what extent the tibial nerve injury model is sensitive to sympathectomy, and whether other manifestations of neuropathic pain, such as spontaneous pain, cold allodynia, contralateral symptoms and (progressive) tactile hyperalgesia develop in the model (Choi et al., 1994; Decosterd et al., 2000; Kim et al., 1997; Koltzenburg et al., 1999; Ma and Woolf, 1996). Interestingly, all transected subjects in the present developed mechanical allodynia. This finding appears to contrast with the spared nerve injury model, in which it was reported that about 20% of transected rats were nonresponders (Erichsen and Blackburn-Munro, 2002). Nevertheless, in the study by Decosterd and Woolf (2000), all transected subjects were responders, so it is still unclear whether the tibial nerve injury model really offers an advantage over the spared nerve injury model in terms of responder rate. Finally, it should be emphasized that body weight developed normally and that, in contrast to some other models, no signs of autotomy were present in the tibial nerve injury model.

The present study observed that tibial nerve injury resulted in the same pattern of gene regulation as obtained in several other rodent models of neuropathic pain (i.e., an up-regulation of galanin and VIP, in addition to a down-regulation of CGRP and substance P; Hökfelt et al., 1994; Honore et al., 2000; Nahin et al., 1994). L5 dorsal root ganglia tissue was selected for this analysis, as preliminary comparative studies using either L4 or L5 dorsal root ganglia tissue had shown that the regulatory effects were more pronounced in the latter tissue. This finding is consistent with the fact that all neuronal cells of L5 dorsal root ganglia are affected by tibial nerve injury, whereas L4 dorsal root ganglia are only partially affected. Quantitative real-time PCR was selected for the gene expression analysis, as it was shown to be very sensitive for the detection of changes in gene expression in dorsal root ganglia using another nerve injury model (Macdonald et al., 2001). This method also detects changes in the expression of cannabinoid CB_1 receptors in the thalamus, and in the expression of metabotropic glutamate $mglu_1$ receptor in dorsal root ganglia in the tibial nerve injury model (Hofmann et al., 2001; Siegling et al., 2001).

The finding that the expression of galanin and VIP mRNA was up-regulated in the tibial nerve injury model is consistent with the previously reported increase of these neuropeptides at the mRNA and/or protein level in other models of neuropathic pain (i.e., partial sciatic nerve injury model, chronic constriction injury model, spinal nerve ligation model, or after sciatic nerve transection; Fukuoka et al., 1998; Ma and Bisby, 1997, 1998, 1999; Nahin et al., 1994; Shi et al., 1993; Zhang et al., 1995, 1998). Those

models revealed the highest level of expression 5–14 days following surgery, and, in the case of VIP, was greatest after sciatic nerve transection, followed by tight ligation and loose ligation of the sciatic nerve, respectively (Shi et al., 1993). The finding that up-regulation of galanin and VIP mRNA expression was greatest around day 5 post-surgery in the tibial nerve injury model is generally consistent with expression data obtained in the other models of neuropathic pain. Similarly, the observed down-regulation of CGRP and substance P mRNA resembles data obtained in other models of neuropathic pain. Thus, mRNA levels of CGRP were decreased after 7–14 days in the chronic constriction injury model, the spinal nerve ligation model, or after transection of the sciatic nerve (Fukuoka et al., 1998; Honore et al., 2000; Nahin et al., 1994; Zhang et al., 1995, 1998). Similarly, down-regulation of substance P after 7–14 days was found at the mRNA level in the chronic constriction injury model, and at the protein level in the spinal nerve ligation model (Nahin et al., 1994; Honore et al., 2000; Hökfelt et al., 1994). Although the functional role of the relatively consistent profile of changes in gene expression regulation across the different models of neuropathic pain is not entirely clear, it has been suggested that down-regulation of excitatory and pro-algesic peptides, like CGRP and substance P, and up-regulation of inhibitory and analgesics peptides, like galanin, are a counter-regulatory mechanism to reduce pain sensitization (Hökfelt et al., 1994).

Pharmacological validation of the tibial nerve injury model was performed with a number of compounds which are currently used for the treatment of neuropathic pain in human patients (for review see: Sindrup and Jensen, 1999). In rodent models, it was previously reported that acute or subacute treatment with morphine, amitriptyline, carbamazepine, baclofen and gabapentin can attenuate behavioral symptoms in various rat models of neuropathic pain (e.g., Abdi et al., 1998; Attal et al., 1991; Decosterd et al., 2000; Desmeules et al., 1993; Erichsen and Blackburn-Munro, 2002; Esser and Sawynok, 1999; Hunter et al., 1997; Koch et al., 1996; LaBuda and Fuchs, 2000; Pan et al., 1999; Patel et al., 2001; Smith et al., 1994). However, not all behavioral symptoms of neuropathic pain are equally responsive to treatment, and that the efficacy and potency of these drugs appear to vary considerably across different models. In general, it appears that the tibial nerve injury model is sensitive to various analgesics with different mechanisms of action, but the efficacy of these compounds was in some cases relatively weak, and obtained only at moderate to high doses. This relative lack of sensitivity to pharmacotherapy is consistent with clinical experience with these compounds (reviewed by Martin and Eisenach, 2001; Sindrup and Jensen, 1999), and underscores the high medical need for effective treatment. Further experiments are needed to clarify whether the efficacy of the currently tested compounds may change after repeated administration, and whether other behavioral endpoints show a different sensitivity to pharmacological treatment. Interestingly, Erichsen and Blackburn-Munro (2002) recently reported that acute treatment with morphine and gabapentin induced a similar degree of efficacy against mechanical allodynia in the spared nerve injury model. In the present study, we found that morphine, but not gabapentin, was able to reverse mechanical allodynia. Although it remains unclear whether the apparent insensitivity of the tibial nerve injury model to gabapentin points to a differential sensitivity of both models to pharmacological treatment, it should be noted that Erichsen and Blackburn-Munro (2002) performed a time-dependency study with gabapentin, and that they found that the anti-allodynic effect was not yet present when tested 1 h after administration (the injection-test interval in the present study). Therefore, it is possible that the apparent lack of sensitivity of the tibial nerve injury to gabapentin is due to an inappropriate injection-test interval. On the other hand, in a preliminary study, in which the effects of gabapentin (100–400 mg/kg) were tested 4 h after oral administration, no anti-allodynic effect could be observed. In addition, it should be noted that gabapentin was reported to be only partially active against tactile allodynia in other models of neuropathic pain, even if tested after longer injection-test intervals (e.g., Kayser and Christensen, 2000; Xiao and Bennett, 1996).

In summary, it appears that the tibial nerve injury model shares a number of behavioral, molecular and pharmacological features with the frequently used ligation models and with the more recently introduced models based on transection of one or more of the branches of the sciatic nerve. The finding that the tibial nerve injury model shows a robust and persistent mechanical allodynia, which is present in all subjects and sensitive to a number of established analgesics and which shows a gene expression profile compatible with that obtained in other models of neuropathic pain, suggests that it offers a surgically uncomplicated model for the study of neuropathic pain.

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