ORIGINAL ARTICLE

Antinociceptive action of carbamazepine on thermal hypersensitive pain at spinal level in a rat model of adjuvant-induced chronic inflammation

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Abstract

Purpose Systemic carbamazepine, a voltage-gated sodium channel blocker, has been reported to dose-dependently reduce inflammatory hyperalgesia. However, the antinociceptive effects of carbamazepine on the spinal cord in inflammatory conditions are unclear. The aim of the present study was to evaluate the antinociceptive effects of carbamazepine on the spinal cord in a chronic inflammatory condition.

Methods In Sprague-Dawley rats, a chronic inflammatory condition was induced by complete Freund's adjuvant (CFA) inoculation into the tail. Tail flick (TF) latencies were measured following intraperitoneal carbamazepine, or intrathecal carbamazepine or tetrodotoxin injection in intact rats and in the chronic inflammatory rats. From the values of TF latency at 60 min after drug injection, the effective dose required to produce 50% response (ED_{50}) of each drug was derived.

Results Carbamazepine attenuated thermal responses with both systemic and intrathecal administration. The effect was more evident in rats with chronic inflammation than in intact rats; the ED_{50}s of intraperitoneal carbamazepine in intact and inflamed rats were 12.39 and 1.54 mg/kg, and those of intrathecal carbamazepine were 0.311 and 0.048 nmol, respectively. Intrathecal tetrodotoxin also clearly inhibited the response, with ED_{50}s of 1.006 pmol in intact rats and 0.310 pmol in inflamed rats. The relative potencies of intrathecal carbamazepine versus tetrodotoxin for inhibition were approximately 1:150–1:300 in intact and inflamed rats.

Conclusion These results indicate that the inhibition of voltage-gated sodium channels, at least tetrodotoxin-sensitive channels, may contribute to the antinociceptive effect of carbamazepine on CFA-induced inflammatory pain, since lower doses of intrathecal carbamazepine and tetrodotoxin attenuated thermal responses to a greater extent in inflamed rats than in intact rats.

Keywords Chronic inflammatory pain · Voltage-gated sodium channel · Carbamazepine · Tetrodotoxin · Complete Freund's adjuvant

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Introduction

Chronic inflammatory pain occurs after the primary cause has subsided, and is caused by infection or other inflammatory processes. Inflammatory mediators increase the sensitivity and excitability of nociceptors (peripheral sensitization) and cause hyperalgesia and allodynia in peripheral tissues. In chronic inflammatory pain, increased sensitivity in the central nervous system (central sensitization) also causes hyperalgesia and allodynia [1].

Voltage-gated sodium channels (VGSCs or Na_v) play a major role in determining the excitability properties of peripheral and central neurons and affect the progress of

inflammatory and neuropathic pain. Clinical and experimental data suggest that changes in VGSCs may play a role in inflammatory pain, and sodium-channel blockers have therapeutic potential in the management of such pain [2]. Carbamazepine, a sodium-channel blocker, has been used for the treatment of neuropathic pain conditions including trigeminal neuralgia, diabetic neuropathy, and post-herpetic neuralgia [3]. Moreover, it has been reported that systemic carbamazepine dose-dependently reduces inflammatory hyperalgesia in rats [4].

The inhibition of VGSCs in the brain [5], the γ-aminobutyric acid (GABA) ergic inhibitory modulation of pain transmission at central adrenergic α_2-receptors [6], and the activation of peripheral adrenergic α_2-receptors [7] are presumed to participate in the antinociceptive action of carbamazepine. However, the antinociceptive effects of carbamazepine at the spinal level under inflammatory conditions are still unclear. The aim of this study was to ascertain the antinociceptive effects of systemic and intrathecally administered carbamazepine and to elucidate their actions on spinal VGSCs compared with those of tetrodotoxin, a selective VGSC blocker, in rats with inflammatory pain.

In this study, we compared the antinociceptive effects of drugs by measuring tail flick (TF) latencies following drug administration in intact rats and rats in which chronic inflammation was induced by complete Freund's adjuvant (CFA) inoculation into the tail.

Materials and methods

With the approval of the animal care and use committee of Kinki University Faculty of Medicine, Sprague-Dawley rats were utilized for the experiments. The animals were bred at the Life Science Research Institute, Kinki University School of Medicine, were maintained under controlled conditions (temperature $23 \pm 0.5°C$; humidity 55%; 12/12 h light/dark cycle), and were fed a commercial diet of CE-2 (Clea Japan, Tokyo, Japan), with tap water ad libitum. Experiments were performed between 13:00 and 17:00 under controlled conditions (temperature $23 \pm 0.5°C$).

Animal preparations

The subarachnoid space was cannulated with a polytetrafluoroethylene (PTFE)-lined polyethylene tube (0.3-mm outside diameter [OD] and 0.11-mm internal diameter [ID]; Microspinal Catheter; Hakko, Nagano, Japan) by application of the modified method of Jensen and Yaksh [8]. In brief, 8-week-old rats were anesthetized with an intraperitoneal injection of pentobarbital sodium. The catheter was passed through a slit in the atlanto-occipital membrane and extended 11 cm caudally to the level of the lumbar spinal cord. The distal end of the catheter was fixed in the subcutaneous tissue to avoid dislocation of the catheter. One week later, rats that exhibited any evidence of sensory or motor dysfunction were excluded from the study.

Upon completion of the experimental procedures, 3 of the catheterized animals were killed by intraperitoneal injection of an overdose of pentobarbital sodium after an intrathecal infusion of 20 μl of indigo carmine through the indwelling intrathecal catheter, and the animals' spines were dissected. On the removal of vertebral bone, the catheter tips were found to be located between L2 and L4 and the dye was distributed from Th12 to the caudal spinal cord.

Tail flick test

The TF test was used to evaluate the antinociceptive effects of the drugs. A Tail-Flick Unit (Model 7360; Ugo Basile, Varese, Italy) was utilized, and the TF latency was measured according to the method of Takasugi et al. [9]. In brief, each rat was placed in a plastic box (22 × 6.5 × 6.5 cm) that had 2 holes on the front wall for oxygen and anesthetic gases and for gas sampling and a hole in the distal wall through which the tail protruded. Under inhalational anesthesia with 1% isoflurane in oxygen, for elimination of the decrease in TF latency due to the learning effect in conscious animals [9], a radiant heat intensity setting of IR20 (161.5 mW/cm^2) was used. Different points along the distal 5–6 cm of the tail were exposed to heat, and a 10-s cut-off was used to minimize the risk of tissue damage. A 10-s interval was maintained between measurements. The mean of the last 5 TF latencies of 7 consecutive measurements was used as the representative value.

The TF latency was converted to represent the maximum possible effect (MPE) according to the following formula:

$$\%MPE = [(\text{test latency}) - (\text{baseline latency})/(\text{cut-off time}) - (\text{baseline latency})] \times 100.$$

Drugs

Carbamazepine, tetrodotoxin, and dimethyl sulfoxide (DMSO) were purchased from Wako Pure Chemical Industries (Osaka, Japan), and CFA, which contained a suspension of *Mycobacterium tuberculosis* H37Ra (an avirulent strain) in a mixture of paraffin oil and mannide monooleate, was purchased from Difco Laboratories (Detroit, MI, USA). Carbamazepine and tetrodotoxin were dissolved in DMSO and normal saline, respectively. CFA was also suspended in normal saline.

In preliminary experiments, intraperitoneal doses of >100 mg/kg carbamazepine in CFA-inflamed rats were fatal, and doses of lumbar intrathecal carbamazepine >5 nmol and tetrodotoxin >10 pmol resulted in motor

paralysis or were fatal in a considerable number of rats. In this study, carbamazepine was administered at doses of 3, 10, 30, and 100 mg/kg i.p. to intact rats and at doses of 3, 10, and 30 mg/kg i.p. to inflamed rats, and carbamazepine and tetrodotoxin for intrathecal administration were prepared at concentrations of 0.1, 0.4, 1.3, and 4.2 nmol/20 μl, and 0.2, 0.6, 2, and 6 pmol/20 μl, respectively.

Adjuvant-induced chronic inflammatory pain model

CFA-induced inflamed rats were utilized for the chronic inflammatory pain model. CFA was inoculated into the tail. Prior to the following experiments, we ascertained histological changes and alteration of TF latencies following CFA inoculation in 9-week-old rats.

For histological examination, 12 rats were inoculated with 5 μg/10 μl of CFA, and 4 rats, serving as controls, were injected with 10 μl of normal saline. Injections were given subcutaneously 5 cm proximal to the tip of the tail. At 3, 7, 14, and 21 days post-inoculation, 3 rats in the CFA group and 1 rat in the control group were killed by an intraperitoneal pentobarbital overdose. Sections of the injection site from each animal were subsequently decalcified for 3 days with formic acid. Five-micron-thick paraffin sections were stained with hematoxylin and eosin and examined by optical microscopy.

To evaluate the alteration of TF latencies following CFA inoculation, another 12 rats were used and randomly divided into 2 groups of 6 rats each, a CFA group and a control (normal saline) group. Prior to each experiment, the rats were placed in the boxes and exposed to 1% isoflurane in oxygen for 20 min, and baseline TF latencies were measured. Then, rats in each group were subcutaneously inoculated with 5 μg/ 10 μl CFA or 10 μl normal saline in the tail. TF latencies were measured at 7, 14, and 21 days post-inoculation.

Antinociceptive effect of systemic carbamazepine

Ten-week-old inflamed rats (7 days after CFA inoculation) and 10-week-old intact rats without indwelling intrathecal catheters were used for this part of the study. Intact and CFA groups of 50 rats each were administered carbamazepine intraperitoneally. Rats in each group were randomly divided into 5 subgroups of 10 rats each. The rats were placed in the boxes and exposed to 1% isoflurane in oxygen for 20 min, and baseline TF latencies were measured immediately before carbamazepine injection in the intact group and immediately before CFA inoculation (at the age of 9 weeks) in the CFA group. Following the measurement of pre-injection TF latencies, doses of 0 (DMSO only), 3, 10, 30, or 100 mg/kg of carbamazepine were injected intraperitoneally in each subgroup, and each rat was tested for 60 min at 10-min intervals.

Antinociceptive effects of intrathecal carbamazepine or tetrodotoxin

Ten-week-old inflamed rats (7 days after CFA inoculation) and 10-week-old intact rats with indwelling intrathecal catheters were used for this part of the experiment. Intact and CFA groups of 90 rats each were intrathecally administered carbamazepine or tetrodotoxin. The rats in each group were randomly divided into 5 and 4 subgroups of 10 rats each, receiving intrathecal carbamazepine and tetrodotoxin, respectively. Baseline TF latencies were measured immediately before drug injection in the intact group and immediately before CFA inoculation (at the age of 9 weeks) in the CFA group. Following the measurement of pre-injection TF latencies, each subgroup of rats received either 0 (DMSO), 0.1, 0.4, 1.3, or 4.2 nmol of carbamazepine or 0 (normal saline), 30, 100, or 300 pmol of tetrodotoxin through the lumbar intrathecal catheter, using a micro-syringe (Hamilton® Syringe 1705LT; Hamilton, Reno, NV, USA), and TF latencies were measured for 60 min at 10-min intervals.

Effective doses required to produce 50% response (ED$_{50}$) with intraperitoneal carbamazepine and intrathecal carbamazepine and tetrodotoxin

From the TF latency values (%MPE) at 60 min after each injection of intraperitoneal carbamazepine, intrathecal carbamazepine and tetrodotoxin in intact and chronic inflamed rats, the ED$_{50}$ of each drug was derived from sigmoidal dose response curves to predict the inhibitory effects. The efficacy of drugs in inflamed rats compared with that in intact rats was assessed by the ED$_{50}$ ratio.

Statistical analysis

Data are expressed as means ± standard error. TF latency changes over time were compared using repeated measures analysis of variance (ANOVA), and comparisons among groups were analyzed by ANOVA followed by Bonferroni post-tests or Tukey's multiple comparison test as indicated. Statistical analysis and the determination of ED$_{50}$ were performed using Prism 5 for Windows Ver. 5.01 (Graph-Pad Software, San Diego, CA, USA). P values of <0.05 were considered statistically significant.

Results

Adjuvant-induced chronic inflammatory pain model

Histological findings following the subcutaneous injection of CFA are shown in Fig. 1. No notable histological

Fig. 1 Histological studies of H&E-stained sections of the injection sites in the rat tail. At 7 days post-inoculation, tissue at the injection site from rats injected with normal saline (*NS*) showed no notable pathological changes (**a**). Sections of the injection site in rats inoculated with complete Freund's adjuvant (*CFA*) at 3, 7, 14, and 21 days (*d*) post-inoculation (**b–e**). Granulomatous lesions around the oil drops (*arrows* in **b**, **c**, and **d**) in the subcutaneous stroma and inflammatory cell infiltrations were observed throughout the observation period in the sections obtained from the CFA-inoculated rats. At 21 days post-inoculation, chronic inflammatory changes, including fibrosis and lymphocyte infiltration, had developed. *Scale* 300 μm
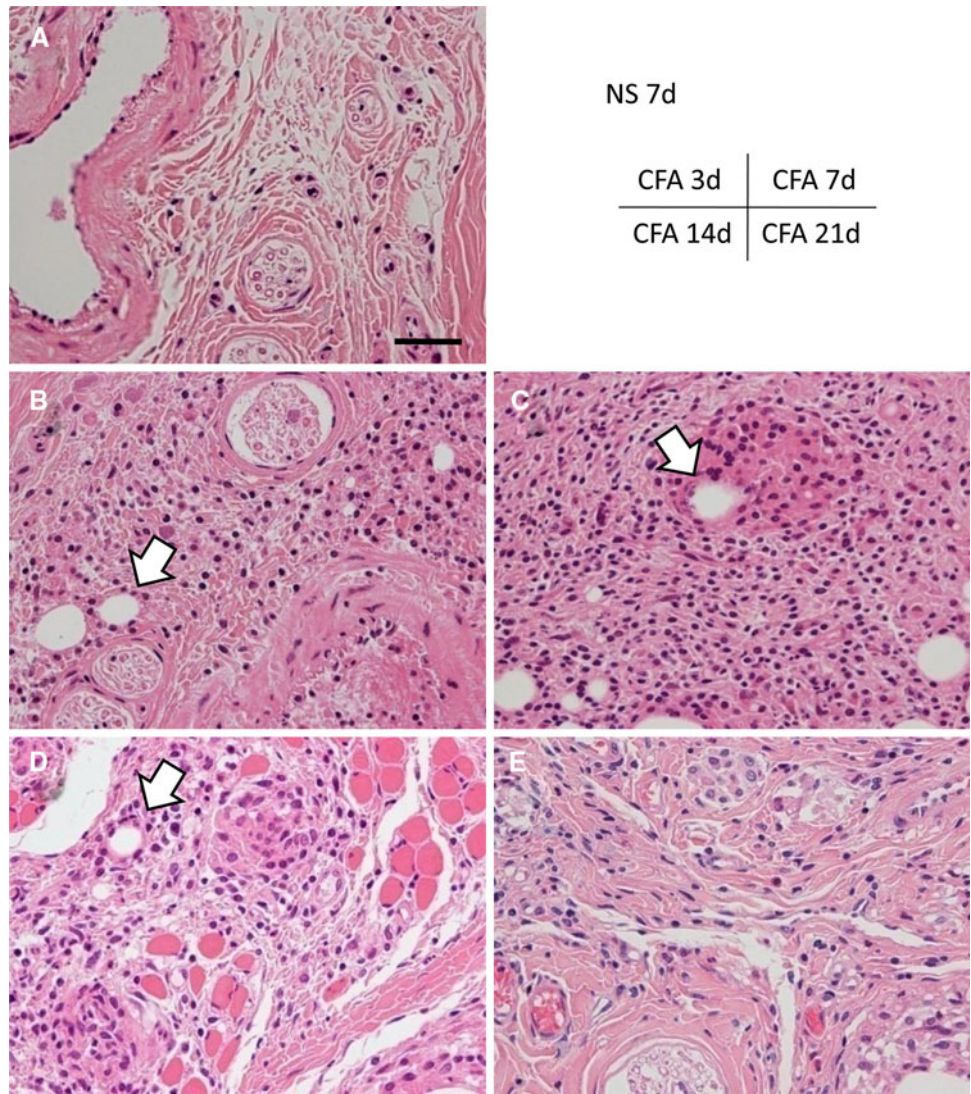
changes were observed throughout the observation period in the sections obtained from rats injected with normal saline. In contrast, inflammatory cell infiltration (of lymphocytes, macrophages, neutrophils, and eosinophils) was observed throughout the observation period in the sections obtained from CFA-inoculated rats. At 3 days post-inoculation, CFA injection sites demonstrated acute interstitial inflammatory changes consisting of neutrophil accumulation and necrosis. At 7 days post-inoculation, inflammatory reactions had lessened, although granulomatous lesions were observed. These consisted of macrophages and neutrophils around the oil drops in the subcutaneous stroma, and evidence of inflammatory cell infiltration into muscle fibers. At 14 days post-inoculation, macrophage infiltration had spread throughout the entire stroma. At 21 days post-inoculation, macrophage infiltration had resolved and chronic inflammatory changes, including fibrosis and lymphocyte infiltration, had developed.

Figure 2 shows alterations of TF latencies (%MPE) during the observation period following the subcutaneous injection of CFA or normal saline into the tail. Mean TF latencies in the normal saline group remained stable, while those in the CFA group shortened significantly from 7 days until 21 days post-inoculation ($P < 0.01$).

In all these drug experiments, CFA inoculation produced significantly shortened TF latencies 7–14 days after the CFA injection (3.5 ± 0.5 s: mean $\pm$ SD) compared to baseline values (5.3 ± 0.3 s), at predicted skin temperatures of 45.2 ± 4.1 and $49.3 \pm 2.6°C$ [9], respectively.

Antinociceptive effect of systemic carbamazepine

TF latencies were not altered for 60 min after vehicle (DMSO) administration in either intact or inflamed rats. With intraperitoneal carbamazepine injection in intact rats, significant prolongations of TF latency were seen after 20,

30, and 60 min in the 100, 30, and 10 mg/kg dose groups, respectively, compared to the vehicle group, while 3 mg/kg carbamazepine injected intraperitoneally in intact rats did not alter TF latencies. In contrast, TF latencies were significantly prolonged with all doses of carbamazepine injected intraperitoneally in inflamed rats at ≥ 10 min after injection, compared to the vehicle group (Fig. 3a, b).

Antinociceptive effects of intrathecal carbamazepine or tetrodotoxin

No rat showed any sign of respiratory distress following the administration of any of the different doses of intrathecal carbamazepine or tetrodotoxin. Higher doses of intrathecal carbamazepine (4.2-nmol) and tetrodotoxin (6-pmol) restricted locomotion due to a sedative effect and motor
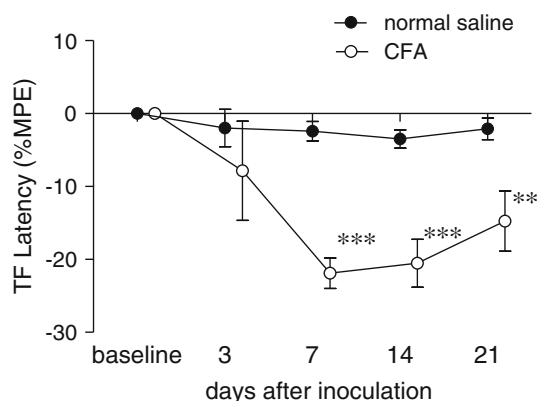

Fig. 2 Change in tail flick (*TF*) latencies (% maximum possible effect [*MPE*]) following subcutaneous injection of complete Freund's adjuvant (CFA) or normal saline into the rat tail. *Closed circles* indicate the normal saline group and *open circles* indicate the CFA group. Data are presented as means ± SE ($n = 12$). **$p < 0.01$, ***$p < 0.001$ versus baseline (Tukey's multiple comparison test)

impairment, respectively, but with low doses, no symptom of motor impairment or paralysis was revealed.

TF latencies were not altered for 60 min after intrathecal vehicle (DMSO or normal saline) administration in either intact or inflamed rats.

With intrathecal carbamazepine injection to intact rats, a 4.2-nmol dose produced significant prolongation of TF latencies immediately after administration compared with those in vehicle-administered rats. Although 0.4- and 1.3-nmol doses also prolonged TF latencies, the %MPE remained around 50%. The 0.1-nmol dose did not alter TF latencies. On the other hand, intrathecal carbamazepine doses of 0.4, 1.3, and 4.2-nmol administered to inflamed rats significantly prolonged TF latencies measured at ≥ 10 min after injection in a similar manner, while the 0.1-nmol dose produced prolongation of TF latencies at ≥ 20 min after injection (Fig. 4a, b).

With intrathecal tetrodotoxin injection in intact rats, the 2- and 6-pmol doses produced prolongation of TF latencies ≥ 10 and 40 min, respectively, after injection compared with those in vehicle-administered rats, while the 0.6-pmol dose did not alter TF latency. In contrast, with intrathecal tetrodotoxin injection in inflamed rats, the 6-pmol dose produced maximal prolongation of TF latencies immediately after administration, while both the 0.6- and 2-pmol doses prolonged TF latencies ≥ 10 min after injection, and the 0.2-pmol dose prolonged TF latencies ≥ 40 min after injection (Fig. 5a, b).

Effective doses required to produce 50% response (ED₅₀) with intraperitoneal carbamazepine and intrathecal carbamazepine and tetrodotoxin

All dose–response curves in inflamed rats shifted to the left compared with those in intact rats with ED$_{50}$s of 12.39 and
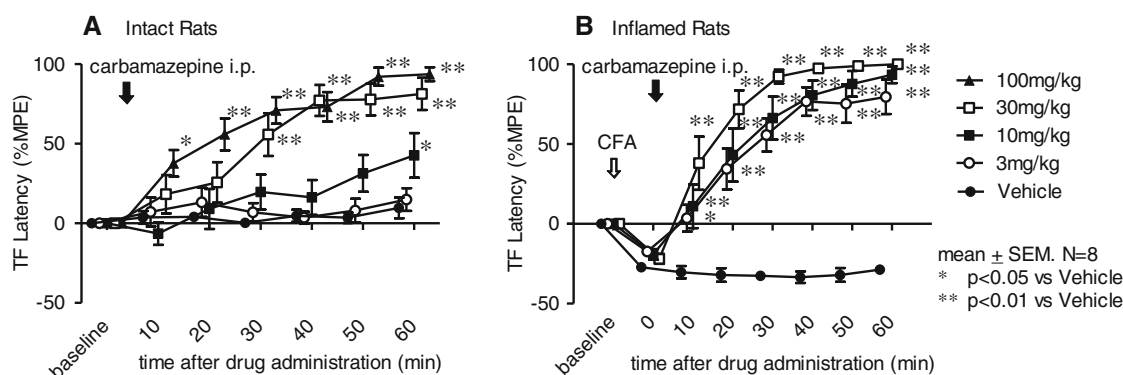

Fig. 3 Changes in tail flick latencies (%MPE) following intraperitoneal (*i.p.*) carbamazepine or vehicle (dimethyl sulfoxide) injection in intact rats (**a**) and inflamed rats induced by complete Freund's adjuvant (CFA) (**b**). *Baseline* indicates values immediately before carbamazepine injection in the intact rats and values pre-CFA

inoculation in inflamed rats. Subcutaneous injection of CFA in the rat tail reduced %MPE at 7–14 days after inoculation. Intraperitoneal injection of vehicle did not alter %MPE in either intact or inflamed rats. Data are presented as means ± SE ($n = 10$). *$p < 0.01$, **$p < 0.05$ versus vehicle (Tukey's multiple comparison test)
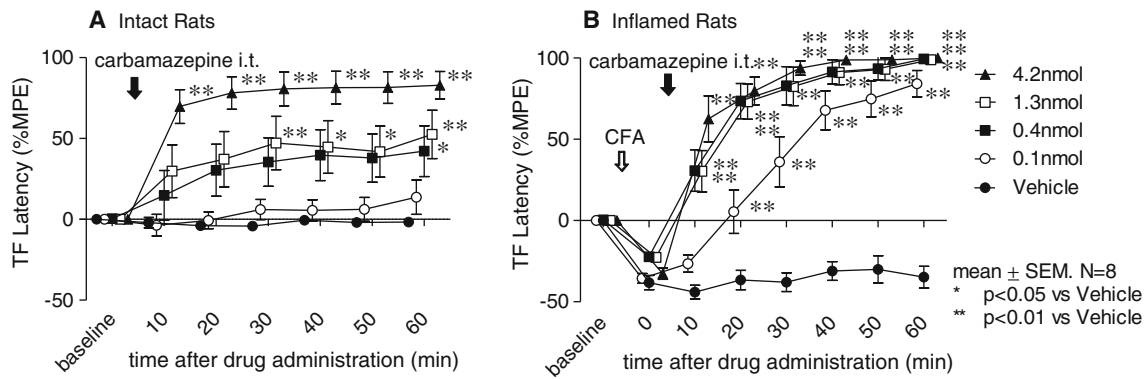
Fig. 4 Changes in tail flick latencies (%MPE) following intrathecal (*i.t.*) carbamazepine or vehicle (dimethyl sulfoxide) injection in intact rats (**a**) and inflamed rats induced by complete Freund's adjuvant (CFA) (**b**). *Baseline* indicates values immediately before carbamazepine injection in intact rats and pre-CFA inoculation values in inflamed rats. Subcutaneous injection of CFA in the rat tail reduced %MPE at 7–14 days after inoculation. Intrathecal injection of vehicle did not alter %MPE in either intact or inflamed rats. Data are presented as means ± SE ($n = 10$). *$p < 0.01$, **$p < 0.05$ versus vehicle (Tukey's multiple comparison test)
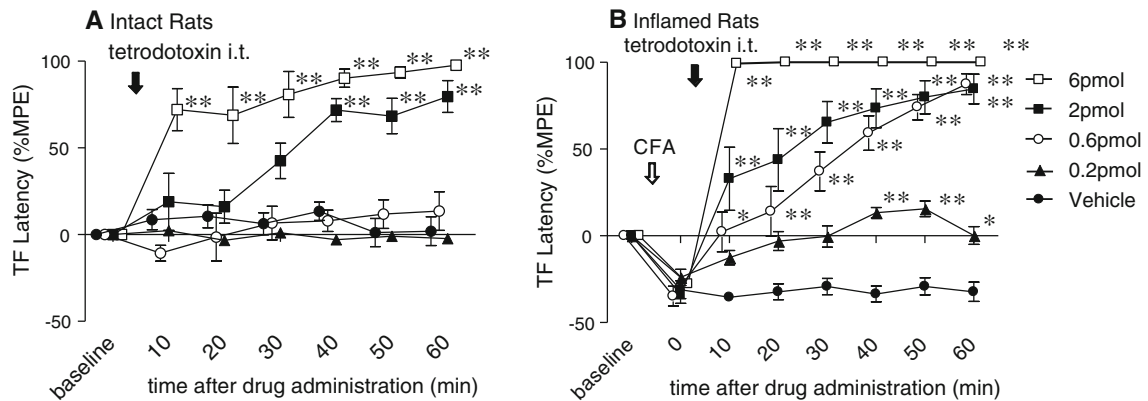

Fig. 5 Changes in tail flick latencies (%MPE) following intrathecal tetrodotoxin or vehicle (normal saline) injection in intact rats (**a**) and inflamed rats induced by complete Freund's adjuvant (CFA) (**b**). *Baseline* indicates values immediately before tetrodotoxin injection in the intact rats and pre-CFA inoculation values in the inflamed rats. CFA injection into the tail reduced %MPE at 7–14 days after inoculation. Intrathecal injection of vehicle did not alter %MPE in either intact or inflamed rats. Data are presented as means ± SE ($n = 10$). *$p < 0.01$, **$p < 0.05$ versus vehicle (Tukey's multiple comparison test)

1.54 mg/kg (ED_{50} ratio for inflamed and intact rats, 0.12) for intraperitoneal carbamazepine, ED_{50}s of 0.311 and 0.048 nmol (0.15) for intrathecal carbamazepine, and ED_{50}s of 1.006 and 0.310 pmol (0.31) for intrathecal tetrodotoxin. The relative potencies of intrathecal tetrodotoxin versus carbamazepine in intact and inflamed conditions, based on the ED_{50} values, were 309 and 155, respectively (Fig. 6; Table 1).

Discussion

In the present study, we demonstrated that carbamazepine attenuated thermal responses by both systemic and intrathecal administration. The antinociceptive effect of carbamazepine was more evident in rats with chronic inflammation than in intact rats. Intrathecal tetrodotoxin also inhibited the thermal response, at lower doses under chronic inflammatory conditions than in the intact rats. The relative inhibitory potencies of intrathecal tetrodotoxin in intact and inflamed rats were approximately 300 and 150 times those of intrathecal carbamazepine, respectively.

In animal models, there is evidence that carbamazepine reduces the noxious stimuli-evoked responses of neurons under inflammatory as well as neuropathic conditions [4, 10–12]. The inhibition of VGSCs in the brain and, consequently, the inhibition of action potentials and glutamergic excitatory neurotransmission have been recognized to be the main antiepileptic action of carbamazepine [5]. The GABAergic inhibitory modulation of pain transmission at central adrenergic α_2-receptors [6] and the activation of peripheral adrenergic α_2-receptors [7] are, at least in part, presumed to participate in the anti-hyperalgesic effects of systemic carbamazepine on inflammatory hyperalgesia.

Fig. 6 Comparison of dose–response curves between intact rats (*closed circles*) and inflamed rats (*open circles*) with intraperitoneal carbamazepine (**a**), intrathecal carbamazepine (**b**), and intrathecal tetrodotoxin (**c**). The ED_{50} was calculated from the sigmoidal dose–response curve (variable slope)
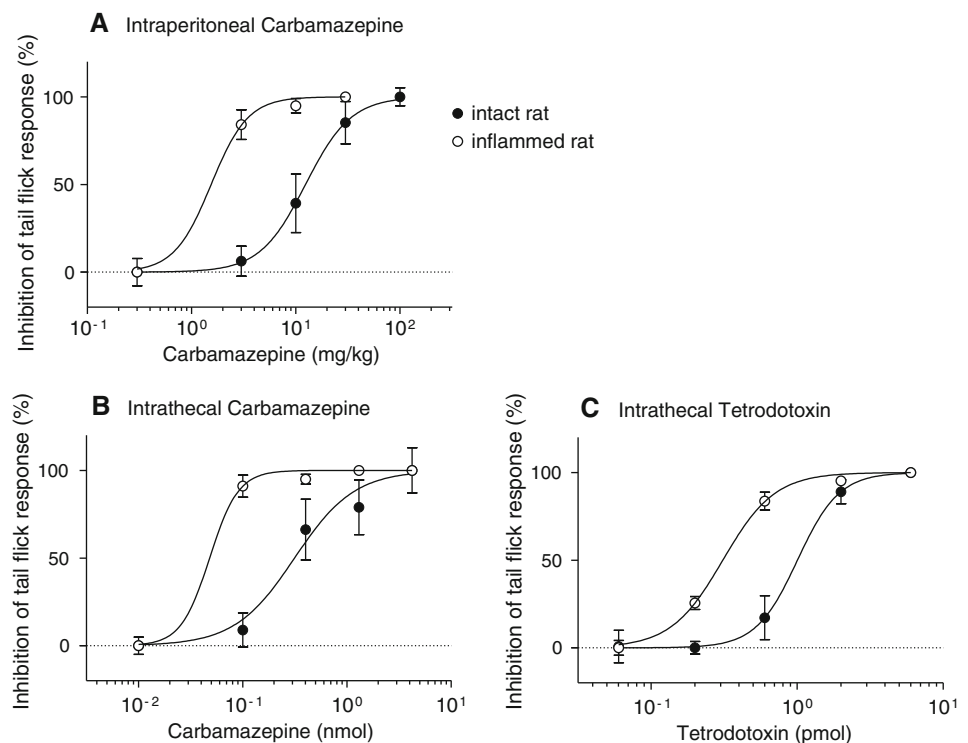

Table 1 ED_{50} of intraperitoneal carbamazepine and intrathecal carbamazepine and tetrodotoxin

	Intact rats	Inflamed rats	ED_{50} ratio (inflamed: intact)
CBZ i.p.	12.39 mg/kg (8.44–18.20)	1.54 mg/kg (0.70–3.41)	0.12
CBZ i.t.	0.311 nmol (0.176–0.561)	0.048 nmol (0.015–0.160)	0.15
TTX i.t.	1.006 pmol (0.767–1.319)	0.310 pmol (0.276–0.349)	0.31
Relative potency (TTX i.t. : CBZ i.t.)	309	155	

The values in parentheses are 95% confidence intervals

ED_{50} the effective dose required to produce 50% response, *CBZ* carbamazepine, *TTX* tetrodotoxin, *i.p.* intraperitoneal, *i.t.* intrathecal

In the present study, the ED_{50} values of intraperitoneal carbamazepine converted to doses for mean body weight (300 g) in intact and inflamed rats were 15.7 and 1.96 μmol, respectively, and the potencies of intrathecal carbamazepine were approximately 50,000 and 40,000 times those of intraperitoneal carbamazepine in intact and inflamed rats, respectively. Concerning morphine, which produces a profound inhibition of noxious stimuli-evoked activity at both the supraspinal and spinal levels [13], the potency of intrathecal morphine was reported to be approximately 1,000 times that of systemic (intraperitoneal) morphine in rats with post-thoracotomy pain [14]. These results may suggest that the inhibition of noxious responses by systemic carbamazepine mainly results from the inhibition of VGSCs at the spinal level by carbamazepine.

Carbamazepine is presumed to inhibit both tetrodotoxin-sensitive (TTX-S) and tetrodotoxin-resistant (TTX-R) VGSCs [15–17]. Although we did not examine the expression of sodium channels in the spinal or dorsal root ganglion (DRG) in our inflammatory rat model, upregulation of the expression of both TTX-S and TTX-R VGSCs in the DRG and dorsal horn neurons has been reported to contribute to inflammatory pain in a variety of animal models [18–21].

Anticonvulsants including carbamazepine typically have much higher binding affinity to the open or inactivated sodium channel than to the resting channel [22–24]. State-dependent compound inhibition of the sodium channel is defined as a profile of a greater block in the inactivated state than in the resting state [25]. In the pain condition, VGSCs are activated (open) and then are rapidly inactivated in response to the depolarization of the plasma membrane of excitable cells, allowing the transient flow of sodium ions [24]. In the present study, with systemic (intraperitoneal) carbamazepine, greater inhibition of noxious responses was evident in rats with chronic inflammation than in intact rats, with an ED_{50} ratio of 0.12. In intact rats, the systemic administration of 30 and 100 mg/kg carbamazepine

gradually reduced the noxious thermal-evoked responses, while 3 and 10 mg/kg doses produced only slight to no inhibition (ED$_{50}$, 12.39 mg/kg). Conversely, the same doses of systemic carbamazepine promptly and significantly inhibited the thermal responses in rats with CFA-induced inflammation (ED$_{50}$, 1.54 mg/kg). We confirmed that intrathecal carbamazepine produced greater inhibition of noxious responses in inflamed rats than in intact rats, with the ED$_{50}$ ratio being 0.15. Thus, in inflammatory conditions, the state-dependent inhibition produced by carbamazepine may play a role in the obvious inhibition of noxious responses by both its systemic and intrathecal administration.

We considered whether such obvious inhibition of noxious responses in inflamed rats by systemic or intrathecal carbamazepine may result from the inhibition of upregulated VGSCs at the spinal level under inflammatory conditions. We investigated the participation of VGSCs in the spinal cord in inflammatory conditions using tetrodotoxin, a selective TTX-S channel blocker. Intrathecal tetrodotoxin also caused greater inhibition of noxious responses in inflamed rats than in intact rats, as did systemic and intrathecal carbamazepine. Thus, the upregulation of VGSCs, at least TTX-S channels, accompanied by chronic inflammatory conditions [18–21] appears to play a role in the inhibition of noxious responses by intrathecal VGSC blockers. Lingamaneni and Hemmings [26] reported that carbamazepine was a potent inhibitor of particular ligand binding to VGSCs, but inhibited ligand binding to GABA$_A$ receptors with minimal effectiveness. Therefore, we could interpret these results to indicate that the upregulation of VGSCs in the spinal cord contributes to the attenuation of thermal responses produced by carbamazepine under inflammatory conditions.

Chapman and Dickenson [10] suggested that a change in the proportion of the type of sodium channels contributing to the relay of noxious messages, or a change in sodium channel sensitivity to carbamazepine contributes to the inhibition of noxious responses. From the results of the present study, we propose that the upregulation of VGSCs, enhancing the binding affinity of VGSC blockers to VGSCs at the spinal level, is a feasible explanation of the significant anti-nociception produced by carbamazepine under inflammatory conditions.

The limitation of the present study is as follows: although the anti-nociceptive effect of carbamazepine under inflammatory conditions was associated with the upregulation of TTX-S channels in the spinal cord, the association with TTX-R channels is still unclear. Several lines of evidence support the upregulation of TTX-R channels, i.e., Na$_v$1.8 and Na$_v$1.9-type channels, in nociceptive sensory neurons or in DRG neurons under inflammatory conditions [21, 27]. A selective Na$_v$1.8 channel blocker, A-803467, is only available for animal experiments in vivo. Further behavioral studies using selective TTX-R channel blockers will clarify the contribution of VGSCs to inflammatory conditions.

In conclusion, we showed that systemic carbamazepine and both intrathecal carbamazepine and tetrodotoxin inhibited noxious responses to thermal stimuli during inflammatory pain conditions. The significant antinociceptive effect of carbamazepine may result from its high binding affinity to upregulated VGSCs at the spinal level in inflammatory conditions.

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