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Title page

Title:

Allodynia and Hyperalgesia in Adjuvant-Induced Arthritic Rats: Time-Course of Their Progression and Efficacy of Analgesics for Them

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Running title page

a) Running title:

Efficacy of Analgesics for Pain in Arthritic Rats

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CFA, complete Freund's adjuvant; PID, post-inoculation day; NSAID, non-steroidal anti-inflammatory drug; ANOVA, analysis of variance.

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ABSTRACT

The complete Freund's adjuvant (CFA)-induced arthritic rat model has extensively served as a laboratory model in the study of arthritic pain. However, the time-courses of allodynia and hyperalgesia and the efficacies of different analgesics have not fully been analyzed in it. Mechanical allodynia, thermal and joint hyperalgesia, and other disease development parameters (body weight, mobility, paw volume and joint stiffness) were measured on post-inoculation day (PID)s 0 - 28 in rats. Acute analgesic efficacies of drugs were evaluated on PID 9 when degrees of allodynia, hyperalgesia and joint stiffness in the ipsilateral paw reached almost the maximum although those in the contralateral paw changed only slightly. In the ipsilateral paw, thermal hyperalgesia reached the maximum on PID 1, whereas mechanical allodynia and joint hyperalgesia progressively developed during the first 7 or 8 days, being tuned in to arthritis development. In the contralateral paw, thermal hyperalgesia never occurred, whereas mechanical allodynia and joint hyperalgesia developed after PID 11. Morphine and tramadol had full efficacies for all the pain parameters tested at sedation-inducing doses. Indomethacin and diclofenac significantly but partially improved thermal and joint hyperalgesia. Amitriptyline significantly reduced thermal and joint hyperalgesia only at sedation-inducing dose. Acetaminophen, carbamazepine and gabapentin had, at the most, very small efficacies. In conclusion, the present study provided integrated information about the time-course of pain and other disease development parameters in the CFA-induced arthritic rats, and clarified acute efficacies of different categories of analgesics for the allodynia and hyperalgesia.

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The complete Freund's adjuvant (CFA)-induced arthritic rat model has extensively served as a laboratory model in the study of arthritic pain. Mechanical allodynia, thermal hyperalgesia and pain on joint movement (joint hyperalgesia), which are prominent features in arthritic pain, have proved to be present in it (Tatsuo et al., 1994; Jasmin et al., 1998; Bertorelli R et al., 1999). The time-courses of their progression after the CFA inoculation, however, have not been fully analyzed. The first aim in the present study, therefore, was to provide integrated information about the time-courses of pain (mechanical allodynia, thermal hyperalgesia and joint hyperalgesia) and other disease development (body weight, mobility, paw volume and joint stiffness) parameters after the CFA inoculation into the single hind paw in rats. Surprisingly, there are no studies that have fully evaluated the analgesic efficacies of different categories of analgesics on mechanical allodynia, thermal hyperalgesia and joint hyperalgesia in the CFA-induced arthritic rat model. The second aim in the present study, therefore, was to investigate this issue. Anti-pyretics (e.g., acetaminophen) and non-steroidal anti-inflammatory drugs (e.g., indomethacin and diclofenac) are the first line drugs in the treatment of arthritic pain. Anti-depressants (e.g., amitriptyline) and anti-epileptics (e.g., carbamazepine and gabapentin) have been frequently used in the treatment of chronic pains in addition to their original use. Opioid receptor agonists (e.g., morphine and tramadol) have strong efficacies for various nociceptive pains. Morphine is a prototype opioid receptor agonist although tramadol has two mechanisms of action (opioid and mono-aminergic). These different categories of agents were tested in the present study. It has recently been considered that analysis of pain-related parameters in arthritic rats should be conducted in the mono-arthritic phase when arthritis is localized around the affected site such as the hind paw, because serious systemic disturbances occur in the poly-arthritic phase, making it difficult to attribute the obtained data exclusively to the effect on the nociceptive system (Millan et al., 1988; Stein et al., 1988; Butler et al., 1992). In the present study, the

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mono-arthritic phase was determined based on the balance between the pain parameters and other disease development parameters.

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Materials and Methods

Animal Preparations. All experiments were performed according to the regulations of the Animal Ethical Committee of Yamanouchi Pharmaceutical Co., Ltd. Male Lewis rats (165 – 220 g; Charles River, Kanagawa, Japan) were kept under conditions of a 13 : 11 h day/night cycle at a constant room temperature. The total number of rats used in the study was 340. Food and water were available ad libitum. Arthritis was induced by the CFA inoculation in the rats. Briefly, 100 mg of Mycobacterium butyricum (Difco Laboratories, Detroit, MI, USA) was thoroughly mixed with 20 ml of paraffin oil. The mixture was, then, autoclaved for 20 min at 120 °C. Each rat was injected in the right footpad (hind paw) with the mixture in a 0.1 ml volume under ether anesthesia. The rats serving as controls were injected with 0.1 ml of saline. In the experiment evaluating the analgesics, eight rats per drug were anesthetized with ether but not treated to serve as naive controls.

Pain Parameters Measurement. Pain and other disease development parameters were measured in the CFA- or saline-treated rats (n=10 for each group) on post inoculation day (PID) 0 (i.e., just before inoculation) and on PIDs 1, 3, 5, 7, 8, 9, 10, 11, 12, 14, 17, 21 and 28. The measurement for pain parameters was conducted for both hind paws. The measurement of mechanical allodynia was performed with a modified method of Gonzalez et al (2000). Briefly, the threshold was measured using the von Frey hairs (Semmes-Weinstein Monofilaments, Stoelting Co, Illinois, USA). The rats were habituated to wire mesh bottom cages prior to the start of the experiment. Static allodynia was tested in the unrestrained rats by touching the plantar surface of the hind paw with von Frey hairs in ascending order of force (1.2, 1.5, 2.0, 3.6, 5.5, 8.5, 12, 15, 29 and 76 g) for up to 6 s or until a paw withdrawal response was elicited. The lowest amount of force required to elicit a response was recorded

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as the withdrawal threshold in log g. Thermal hyperalgesia was assessed using the plantar test (model 7370, Ugo Basile, Varese, Italy) and a modified method of Hargreaves et al (1988). Briefly, the rats were habituated to an apparatus consisting of individual perspex boxes on an elevated glass table. A mobile radiant heat source was located under the table and focused on the hind paw, and the paw withdrawal latencies were defined as the time taken by the rat to remove its hind paw from the heat source. The cut-off point was set at 15 s to prevent tissue damage. The apparatus was calibrated to give a paw withdrawal latency of approximately 10 s in naive rats. The measurement of joint hyperalgesia was performed by a modification of the previously reported method (Rupniak et al., 1997). The body of rats was held from the back with the left palm, and the bending and extension (one after the other and 5 times in each direction) of the ankle within its limits of range of motion were performed with the right fingers. The total number of vocalizations emitted following the manipulation (the bending and extension, 5 times in each direction) was recorded for each paw (the maximum score was 10 for each paw).

Disease Development Parameters Measurement. The scoring of mobility was performed by modifying the evaluation scale reported by Butler et al (1992). Score 6: walks normally; Score 5: walks being protective toward the ipsilateral hind paw (touches the ipsilateral hind paw fully on the floor); Score 4: walks being protective toward the ipsilateral hind paw (touches only the toe of the ipsilateral hind paw on the floor); Score 3: walks being protective toward both hind paws (touches the contralateral hind paw fully on the floor); Score 2: walks being protective toward both hind paws (touches only the toe of the contralateral hind paw on the floor); Score 1: Crawls only using the fore paws; Score 0: Does not move. Paw volumes were measured by volume displacement of electrolyte solution in the plethysmometer (model TK-105, Unicom, Japan). The hind paw was immersed to the junction of the hairy skin, and the volumes were read on a digital display. The scoring of

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joint stiffness was performed as follows. The body of rats was held from the back with the left palm, and the bending and extension (once in each direction) of the ankle within its limits of range of motion were performed with the right fingers. It was confirmed beforehand that there was no restriction of ankle joint movement in the bending and extension manipulations in naive rats, and the scoring was performed according to the evaluation scale reported by Butler et al (1992). Score 2: there were restrictions of full range of movement of the ankle in both bending and extension; Score 1: there was a restriction of full range of movement of the ankle in bending or extension; Score 0: No restriction. The measurements for paw volume and joint stiffness were conducted for both hind paws. All the time-course observations were performed by one of the authors to avoid inter-observer differences. The time-course measurement experiment was carried out by the observer who was not blind to the CFA or saline treatment since the difference between the ipsilateral and contralateral paw was apparent mainly due to the difference in paw volume.

Drugs. Acetaminophen, indomethacin, diclofenac sodium and amitriptyline hydrochloride were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Morphine hydrochloride and carbamazepine were purchased from Takeda Chemical Industries (Osaka, Japan) and Wako Pure Chemical Industries (Osaka, Japan), respectively. Crispin Injection®, which contained 100 mg of tramadol hydrochloride per 2 ml, was purchased from Nippon Shinyaku Co., Ltd. Gabapentin was prepared by Yamanouchi Pharmaceutical Co., Ltd. Acetaminophen, diclofenac and carbamazepine were suspended in 0.5% methylcellulose. Amitriptyline and morphine were dissolved in saline. Gabapentin and indomethacin were dissolved in distilled water and 0.1 M Tris buffer, respectively. Tramadol was diluted with saline. The conditions, under which each drug had proved to exhibit its optimal analgesic effect in other rat pain models, were employed for each drug evaluation in order to evaluate sufficiently the maximum efficacy. Indomethacin was treated for 3 - 30 mg/kg p.o. 1 h prior

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to the pain parameter measurement (Okuyama and Aihara, 1984). Diclofenac was treated for 10 - 100 mg/kg i.p. 30 min prior to measurement (Euchenhofer et al., 1998). Acetaminophen was treated for 100 - 600 mg/kg p.o. 30 min prior to measurement (Granados-Soto et al., 1993). Amitriptyline was treated for 3 - 30 mg/kg i.p. 30 min prior to measurement (Korzeniewska-Rybicka and Plaznik, 1998). Carbamazepine was treated for 20 - 80 mg/kg p.o. 1 h prior to measurement (Nakamura-Craig and Follenfant, 1995). Gabapentin was treated for 30 - 300 mg/kg p.o. 1 h prior to measurement (Field et al., 1999). Morphine was treated for 1 - 10 mg/kg s.c. 30 min prior to measurement (Bertorelli et al., 1999). Tramadol was treated for 9 - 88 mg/kg s.c. 30 min prior to measurement (Giusti et al., 1997). Drug concentrations were calculated according to the base form.

Analgesic Efficacy Evaluation. Thirty-two rats (8 rats per dose and 4 doses per drug) treated with the CFA and another 8 rats as naive controls were used for each drug evaluation. The analgesic effects were evaluated on PID 9, when mechanical allodynia, thermal hyperalgesia, joint hyperalgesia and joint stiffness in the ipsilateral paw reached almost the maximum, although those parameters in the contralateral paw changed only slightly and the systemic disturbance shown by the change of mobility score was small. On the day before evaluation (PID 8), body weight, mechanical allodynia, thermal hyperalgesia and joint hyperalgesia were measured for the 32 rats that were to be used for drug evaluation. The rats were allocated to 4 groups (8 rats per group) such that the differences in the averages of those parameters among the groups became small. The behavioral disturbance was small in the CFA treated rats on PID 9 except that the rats walked being protective only toward the ipsilateral hind paw. During the evaluation of analgesic efficacy, the behavioral abnormalities that might be caused by the non-specific drug effect such as reduction of motion were also checked by observing the animals. All the analgesic effect evaluations and behavioral observations were performed by one of the authors to avoid inter-observer

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differences. All the analgesic effect evaluations and behavioral observations were carried out by the observer who was blind to the drug treatment.

Statistical Analysis. Data were expressed as the mean ± SEM. The time-course curves for mechanical allodynia, thermal hyperalgesia, joint hyperalgesia, body weight and paw volume were subjected to two-way repeated measures analysis of variance (ANOVA) with post-hoc t-test. In experiments for drug evaluation, the difference in scores between the vehicle-treated and naive control groups was analyzed by Student's t-test to confirm significant changes in the pain parameters in the ipsilateral paw. The analgesic effects were analyzed by Dunnett's t-test, and in each case, the drug-treated groups were compared with the vehicle-treated group. In each statistical analysis, the comparison was conducted for paws on the corresponding side. $P < 0.05$ was considered statistically significant.

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Results

Time-Courses of Pain Parameters. The time-course curves for mechanical allodynia were significantly different between the CFA- and saline-treated rats in both hind paws (two-way repeated measures ANOVA). The thresholds in both hind paws in the saline-treated rats remained stable over the observation period. The threshold in the ipsilateral paw in the CFA-treated rats decreased progressively during the first 7 days and reached a plateau that lasted throughout the rest of the observation period. The differences in the ipsilateral threshold between the CFA- and saline-treated rats were statistically significant on PIDs 1 - 28 (post-hoc t test). The threshold in the contralateral paw in the CFA-treated rats remained almost stable until PID 9, decreased progressively from PID 10 to PID 14 and reached a plateau. The differences in the contralateral threshold between the CFA- and saline-treated rats were statistically significant on PIDs 10 - 28. The decrease of threshold in the ipsilateral paw was significantly larger in magnitude than that in the contralateral paw (Fig. 1A). The time-course curves for thermal hyperalgesia were significantly different between the CFA- and saline-treated rats in the ipsilateral hind paw, although those were not between the CFA- and saline-treated rats in the contralateral paw. The latencies in both paws in the saline-treated rats and in the contralateral paw in the CFA-treated rats remained, apart from fluctuations of a small magnitude, stable throughout the observation period. The latency in the ipsilateral paw in the CFA-treated rats decreased markedly on PID 1, showed a rebound on PID 3, and remained decreased during the rest of the observation period. The differences in the ipsilateral latency between the CFA- and saline-treated rats were statistically significant on PIDs 1 - 28 (Fig. 1B). The time-course curves for the joint hyperalgesia were significantly different between the CFA- and

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saline-treated rats in both hind paws. The numbers of vocalization in both paws in the saline-treated rats remained almost zero throughout the observation period. The numbers of vocalization in the ipsilateral paw in the CFA-treated rats increased progressively during the first 8 days and reached a plateau. The differences in the ipsilateral number of vocalization between the CFA- and saline-treated rats were statistically significant on PIDs 1 - 28. The numbers of vocalization for the contralateral paw in the CFA-treated rats remained almost zero until PID 9, increased progressively from PID 10 to PID 17 and reached a plateau. The differences in the contralateral number of vocalization between the CFA- and saline-treated rats were statistically significant on PIDs 11 - 28 (Fig. 1C).

Time-Courses of Other Disease Development Parameters. The time-course curves for body weight between the CFA- and saline-treated rats were significantly different. The CFA-treated rats showed a decreased rate of body weight gain compared to the saline-treated rats. The difference in the body weight between the CFA- and saline-treated rats grew almost constantly from PID 3 to PID 28. The differences in body weight between the CFA- and saline-treated rats were statistically significant on PIDs 1 - 28 (Fig. 2A). The mobility score in the CFA-treated rats decreased progressively from PID 1 to PID 17 and reached a plateau. The scores during PIDs 7 - 10 remained at around 4, indicating that the rats walked being protective only toward the ipsilateral hind paw and that the behavioral disturbance was small on those days (Fig. 2B). The time-course curves for hind paw volume between the CFA- and saline-treated rats were significantly different in both paws. The paw volumes in both paws in the saline-treated rats increased slightly but steadily throughout the observation period. The volume of ipsilateral paw in the CFA-treated rats increased progressively during the first 5 days, reached a first plateau, increased again progressively from PID 11 to PID 14 and reached a second plateau. The differences in the ipsilateral paw volume between the

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CFA- and saline-treated rats were statistically significant on PIDs 1 - 28. Paw volume of the contralateral paw in the CFA-treated rats for once decreased on PID 1, then increased slightly but steadily from PID 1 to PID 10, increased rapidly from PID 11 to PID 17 and reached a plateau. The differences in the contralateral paw volume between the CFA- and saline-treated rats were statistically significant on PIDs 1 - 8 and PIDs 11 - 28. The increase of volume in the ipsilateral paw was significantly larger in magnitude than that in the contralateral paw (Fig. 2C). There was no restriction on the full range movement of joints in any of rats on PID 0, and the joints of the saline-treated rats showed no restriction throughout the observation period. Joint stiffness score in the ipsilateral paw in the CFA-treated rats increased progressively during the first 8 days and reached a plateau. The joint stiffness score in the contralateral paw in the CFA-treated rats remained almost zero until PID 10, increased progressively from PID 11 to PID 17 and reached a plateau (Fig. 2D).

Efficacies of Analgesics. Non-steroidal anti-inflammatory drugs (NSAIDs) indomethacin and diclofenac did not significantly reduce mechanical allodynia (Figs. 3A and B). They significantly reduced thermal hyperalgesia at 30 mg/kg p.o. for indomethacin (Fig. 3C) and 100 mg/kg i.p. for diclofenac (Fig. 3D). They significantly reduced joint hyperalgesia at 10 and 30 mg/kg p.o. for indomethacin (Fig. 3E) and 100 mg/kg i.p. for diclofenac (Fig. 3F). Neither indomethacin nor diclofenac affected scores in the contralateral paw (Figs. 3A - F). Neither of them influenced the behavior of rats, at least, during the observation period. An anti-pyretic acetaminophen did not significantly improve any pain parameter tested. It did not affect scores in the contralateral paw (Figs. 4A, C and E). It did not influence the behavior of rats, at least, during the observation period. An anti-depressant amitriptyline failed to improve mechanical allodynia (Fig. 4B). It, however, significantly and fully reduced thermal hyperalgesia (Fig. 4D) and significantly reduced joint hyperalgesia (Fig. 4F)

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at 30 mg/kg i.p. It did not affect mechanical threshold (Fig. 4B) or numbers of vocalization in the contralateral paw (Fig. 4F), although it significantly increased latency of response to the thermal stimulation in it (Fig. 4D) at 30 mg/kg i.p. It caused reduction of motion in rats at the dose (30 mg/kg i.p.) that showed significant analgesic effects. Anti-epileptics carbamazepine and gabapentin did not reduce mechanical allodynia (Figs. 5A and B). Carbamazepine significantly reduced thermal hyperalgesia at 80 mg/kg p.o. (Fig. 5C). Gabapentin failed to affect thermal hyperalgesia (Fig. 5D). Neither gabapentin nor carbamazepine reduced joint hyperalgesia (Figs. 5E and F). Neither carbamazepine nor gabapentin affected scores in the contralateral paw (Figs. 5A - F). Carbamazepine induced reduction of motion in rats at the dose (80 mg/kg p.o.) that showed a significant effect on thermal hyperalgesia. Opioid receptor agonists morphine and tramadol improved all the pain parameters tested. They recovered all the pain parameters to naive control level at higher doses (Figs. 6A - F). Morphine significantly increased mechanical threshold and latency of response to the thermal stimulation in the contralateral paw (Figs 6A and C), although tramadol significantly increased only the latency of response to the thermal stimulation in it (Fig. 6D). Both morphine and tramadol produced reduction of motion in rats at the doses (10 mg/kg s.c. for morphine, 88 mg/kg s.c. for tramadol) that showed full efficacies in the ankle flexion test.

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Discussion

The present study revealed that there were differences in the time-courses among the pain parameters measured. Thermal hyperalgesia in the ipsilateral paw reached the maximum even on PID 1. It is unlikely that the hyperalgesia on PID 1 is caused mainly by progression of arthritis because ankle stiffness score remains almost normal on that day. It is possible that the hyperalgesia is attributable mainly to the CFA-induced acute inflammation in the ipsilateral paw because paw volume in the ipsilateral paw largely increases on PID 1 despite the absence of increase of ankle stiffness. In contrast to the rapid progression of thermal hyperalgesia in the ipsilateral paw, the degrees of mechanical allodynia and joint hyperalgesia in it remain small on PID 1. Since the time-courses of mechanical allodynia and joint hyperalgesia in the ipsilateral paw were tuned in to that of joint stiffness in it, it is possible that the development of arthritis is needed to produce full mechanical allodynia and joint hyperalgesia. There were also differences in the time-courses and maximum pain scores between the ipsilateral and contralateral paws. Thermal hyperalgesia never occurred in the contralateral paw even though paw volume and joint stiffness scores in it increased progressively from PID 11 to PID 17. On the other hand, the time-courses of mechanical allodynia and joint hyperalgesia in the contralateral paw concurred with the progression of the paw volume and joint stiffness in it. The absence of thermal hyperalgesia in the contralateral paw in the CFA-induced arthritic rats is in accordance with the result of the previous study (Bertorelli et al., 1999). The reason for the absence in the contralateral paw remains to be clarified, although the inflammation induced directly by the CFA may have a critical role in the induction of thermal hyperalgesia that occurs only in the ipsilateral paw. The maximum scores of joint hyperalgesia and joint stiffness were almost equal between the ipsilateral and contralateral paws, suggesting that the CFA-induced conralateral arthritis has a

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potential to cause full joint hyperalgesia without the inflammation induced directly by the CFA. On the other hand, the maximum score of mechanical allodynia in the contralateral paw was apparently smaller than that in the ipsilateral paw. It is unlikely that the degree of inflammation is responsible for the difference because there exists a clear difference in the mechanical allodynia score even when paw volume scores are almost equal between the ipsilateral and contralateral paws (for example, PID 9 in the ipsilateral paw versus PID 17 in the contralateral paw). It is possible that some factors involved in the CFA-induced direct inflammation have a critical role also in the induction of full mechanical allodynia.

An anti-pyretic acetaminophen, the first line drug in the treatment of arthritic pain, unexpectedly had rather small efficacies in reducing allodynia or hyperalgesia in the present study. The weak effect on mechanical allodynia may reflect the recently published report which shows that acetaminophen is poorly active, giving only maximum 30 % reversal of mechanical hyperalgesia assessed with the paw pressure test in the CFA-induced arthritic rats (Burgess et al., 2000). The weak effect on thermal hyperalgesia is in accordance with the previous report which shows that efficacy of acetaminophen in reducing thermal hyperalgesia measured with the plantar test is very small at 300 mg/kg p.o. in the CFA-induced arthritic rats (Bertorelli et al., 1999). Since acetaminophen has been reported to be effective in the test using electrical stimulation to the foot in the CFA-induced arthritic rats (Okuyama and Aihara, 1984), its effect seems test (stimulation)-dependent. The present study, however, suggested that the acute dosing of acetaminophen was not so effective in reducing mechanical allodynia, thermal hyperalgesia or joint hyperalgesia in the CFA-induced arthritic rats. NSAIDs, other first line drugs in the treatment of arthritic pain, were also unexpectedly ineffective in reducing mechanical allodynia in the present study. This result is inconsistent with the previous report which shows that NSAIDs are effective in reducing mechanical hyperalgesia assessed with the paw pressure test in the CFA-induced arthritic rats (Attal et al.,

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1988; Chau and Weichman, 1989). This discrepancy may be attributable to the different methods employed to measure mechanical thresholds, i.e., the paw pressure test stimulates joints and surrounding deep tissues, whereas the von Frey hairs used in the present study excite mainly cutaneous tissues. The limited efficacy of NSAIDs in the plantar test and ankle flexion test observed in the present study is in accordance with results of the previous studies, in which indomethacin partially reduces number of vocalization in the ankle flexion test at 10 mg/kg p.o. (Rupniak et al., 1997) or reduces thermal hyperalgesia measured with the plantar test at 3 mg/kg i.p. with limited efficacy (Bertorelli et al., 1999) in the CFA-induced arthritic rats. The limited efficacies of anti-pyretic and NSAIDs observed in the present study may reflect the clinical situation that their efficacies are not fully satisfactory although they are preferred analgesics for the management of arthritic pain. It should, however, be noted that they are chronically used in the clinic and their anti-inflammatory actions are considered to contribute to their therapeutic effects. Since the anti-inflammatory effect has not been evaluated in the present study, the results obtained may underestimate their potential in the treatment of arthritic pain.

An anti-depressant amitriptyline induced a full recovery of thermal hyperalgesia and partially attenuated joint hyperalgesia only at the sedative dose, although it was ineffective for mechanical allodynia. There are few reports concerning the analgesic efficacy following single administration of anti-depressants in the CFA-induced arthritic rats, although it has been reported that chronic (4 weeks) administration of amitriptyline or imipramine does not modify the mechanical threshold measured with the paw pressure test (Butler et al., 1985). When the survey is extended to other inflammatory pain models, it has been reported that intrathecal amitriptyline reverses thermal hyperalgesia measured with the plantar test in the carrageenan-induced inflamed rats (Eisenach and Gebhart, 1995). Most animal studies concerning analgesic effects of anti-depressants have been performed in acute pain models

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such as tail flick test and acetic acid-induced writhing test, and it has been shown that anti-depressants are most potent in the chemical test such as the rat acetic acid-induced writhing test, but ineffective in tests using thermal or mechanical stimulus with some exceptions (Korzeniewska-Rybicka and Plaznik, 1998). It was interesting that amitriptyline was most sensitive to the plantar test which used thermal stimulus in the present study, being different from the results in the acute pain models. The reason for this discrepancy remains to be studied.

A conventional anti-epileptic carbamazepine or new-generation one gabapentin was almost completely ineffective in all of the pain parameters tested. These negative results contradict results of the previous studies which have shown that gabapentin attenuates mechanical hyperalgesia measured with the paw pressure test in the carrageenan-induced inflamed rats (Field et al., 1997) or reduces thermal hyperalgesia measured with the plantar test in the kaolin/carrageenan-induced inflamed rats (Lu and Westlund, 1999). One possible reason for this discrepancy is the difference of animal models used. The CFA-induced arthritic pain model, a chronic inflammatory pain model, was used in the present study, although acute inflammatory pain models such as the carrageenan-induced inflamed rat model were used in the previous study. It has recently been reported that gabapentin is only weakly active against mechanical hyperalgesia measured with the paw pressure test even at flaccidity-inducing doses in the CFA-induced arthritic rats (Patel et al., 2001). It is conceivable that different pain mechanisms underlie the CFA-induced arthritis model and acute inflammatory pain models.

Opioid receptor agonists morphine and tramadol fully recovered all the pain parameters tested to the naive control level in the present study. Morphine has proved to exert strong analgesic effects in the CFA-induced arthritic rats when assessed, in most cases, with the plantar test (Bertorelli et al., 1999; Fraser et al., 2000) or paw pressure test (Maldonado et al.,

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1994; Burgess et al., 2000). The present study further showed that morphine exerted strong analgesic effects also on mechanical allodynia and joint hyperalgesia, and that a less potent opioid receptor agonist tramadol, like morphine, exerted full efficacies in all the pain parameters tested. The present study suggested that opioid receptor agonists had full efficacies in reducing mechanical allodynia, thermal hyperalgesia and joint hyperalgesia in the CFA-induced arthritic rats. It should be, however, noted that both morphine and tramadol induced reduction of motion in rats at the doses that showed full efficacies. This might reflect the clinical situation that the efficacy of opioid receptor agonists is strong for various nociceptive pains, although their side effects are the limits of their usability.

In conclusion, the present study provided integrated information about the time-course of pain and other disease development parameters in the CFA-induced arthritic rats, and clarified acute efficacies of different categories of analgesics in reducing the allodynia and hyperalgesia by the evaluation on PID 9 (mono-arthritic phase).

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Footnotes

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Legends for figures

Fig. 1. The time-courses of pain parameters (A: mechanical allodynia, B: thermal hyperalgesia, C: joint hyperalgesia). The parameters were measured on post inoculation day (PID) 0, or on the verge of the adjuvant- or saline-inoculation into the right hind paw, and on PIDs 1, 3, 5, 7, 8, 9, 10, 11, 12, 14, 17, 21 and 28. Mechanical allodynia was measured by the von Frey hair test and represented in log g. Thermal hyperalgesia was assessed with the plantar test and represented in s. Joint hyperalgesia was assessed by the ankle flexion test and represented in the numbers of vocalizations caused by bending and extension of the ankle (5 times in each direction). All results were expressed as the mean ± SEM in 10 rats per group. An open circle or square represents the mean in the right (ipsilateral) or left (contralateral) hind paw, respectively, in the saline-treated rats, and a closed circle or square represents the mean in the ipsilateral or contralateral hind paw, respectively, in the adjuvant-treated rats.

Fig. 2. The time-course of disease development parameters (A: body weight, B: mobility, C: paw volume, D: joint stiffness). The parameters were measured on post inoculation day (PID) 0, or on the verge of the adjuvant- or saline-inoculation into the right hind paw, and on PIDs 1, 3, 5, 7, 8, 9, 10, 11, 12, 14, 17, 21 and 28. The scoring of mobility was performed by modification of the evaluation scale reported by Butler et al (1992). The paw volume was measured by volume displacement of an electrolyte solution in a plethysmometer and represented in ml. The scoring of joint stiffness was performed by the evaluation scale reported by Butler et al (1992). All results were expressed as the mean ± SEM in 10 rats per group. In Figs. 2A and 2B, an open or closed circle represents the mean in the saline- or

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adjuvant-treated rats. In Figs. 2C and 2D, an open circle or square represents the mean in the right (ipsilateral) or left (contralateral) hind paw, respectively, in the saline-treated rats, and a closed circle or square represents the mean in the ipsilateral or contralateral hind paw, respectively, in the adjuvant-treated rats.

Fig. 3. Effects of indomethacin (A, C, E) and diclofenac (B, D, F) on mechanical allodynia, thermal hyperalgesia and joint hyperalgesia in the adjuvant-induced mono-arthritic rats. Indomethacin was orally administered 1 h prior to the measurement of pain parameters. Diclofenac was intraperitoneally administered 30 min prior to the measurement of pain parameters. The results were expressed as the mean ± SEM in 8 rats per group. Open and closed columns represent the means in the left (contralateral) and right (ipsilateral) hind paws, respectively, and a vertical bar represents ± SEM. $^{###}P < 0.001$ significantly different from the ipsilateral (right) hind paw in naive control rats (Student's t-test). $*P < 0.05$, $**P < 0.01$ significantly different from the vehicle-treated ipsilateral hind paw (Dunnett test).

Fig. 4. Effects of acetaminophen (A, C, E) and amitriptyline (B, D, F) on mechanical allodynia, thermal hyperalgesia and joint hyperalgesia in the adjuvant-induced mono-arthritic rats. Acetaminophen was orally administered 30 min prior to the measurement of pain parameters. Amitriptyline was intraperitoneally administered 30 min prior to the measurement of pain parameters. The results were expressed as the mean ± SEM in 8 rats per group. Open and closed columns represent the means in the left (contralateral) and right (ipsilateral) hind paws, respectively, and a vertical bar represents ± SEM. $^{###}P < 0.001$ significantly different from the ipsilateral (right) hind paw in naive control rats (Student's t-test). $**P < 0.01$, $***P < 0.001$ significantly different from the vehicle-treated ipsilateral

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hind paw (Dunnett's t-test). $^{!!!}P < 0.001$ significantly different from the vehicle-treated contralateral hind paw (Dunnett's t-test). Note that amitriptyline caused reduction of motion in rats at the dose (30 mg/kg i.p.) that showed significant analgesic effects.

Fig. 5. Effects of carbamazepine (A, C, E) and gabapentin (B, D, F) on mechanical allodynia, thermal hyperalgesia and joint hyperalgesia in the adjuvant-induced mono-arthritic rats. Carbamazepine and gabapentin were orally administered 1 h prior to the measurement of pain parameters. The results were expressed as the mean ± SEM in 8 rats per group. Open and closed columns represent the means in the left (contralateral) and right (ipsilateral) hind paws, respectively, and a vertical bar represents ± SEM. $^{###}P < 0.001$ significantly different from the ipsilateral (right) hind paw in naive control rats (Student's t-test). $*P < 0.05$ significantly different from the vehicle-treated ipsilateral hind paw (Dunnett's t-test). Note that carbamazepine induced reduction of motion in rats at the dose (80 mg/kg p.o.) that showed a significant effect on the thermal hyperalgesia.

Fig. 6. Effects of morphine (A, C, E) and tramadol (B, D, F) on mechanical allodynia, thermal hyperalgesia and joint hyperalgesia in the adjuvant-induced arthritic rats. Morphine and tramadol were subcutaneously administered 30 min prior to the measurement of pain parameters. The results were expressed as the mean ± SEM in 8 rats per group. Open and closed columns represent the means in the left (contralateral) and right (ipsilateral) hind paws, respectively, and a vertical bar represents ± SEM. $^{###}P < 0.001$ significantly different from ipsilateral (right) hind paw in naive control rats (Student's t-test). $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ significantly different from the vehicle-treated ipsilateral hind paw (Dunnett's t-test). $^{!}P< 0.01$, $^{!!!}P < 0.001$ significantly different from the vehicle-treated contralateral hind

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paw (Dunnett's t-test). Note that both morphine and tramadol produced reduction of motion in rats at the doses (10 mg/kg s.c. for morphine, 88 mg/kg s.c. for tramadol) that showed full efficacies in the ankle flexion test.

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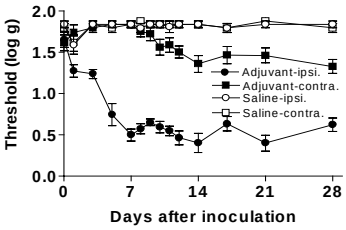
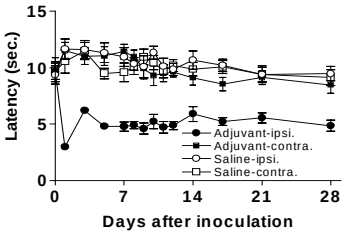
Figure 1

A: Mechanical allodynia



B: Thermal hyperalgesia



C: Joint hyperalgesia

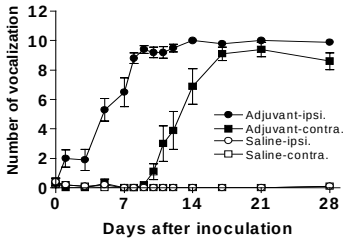


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Figure 2

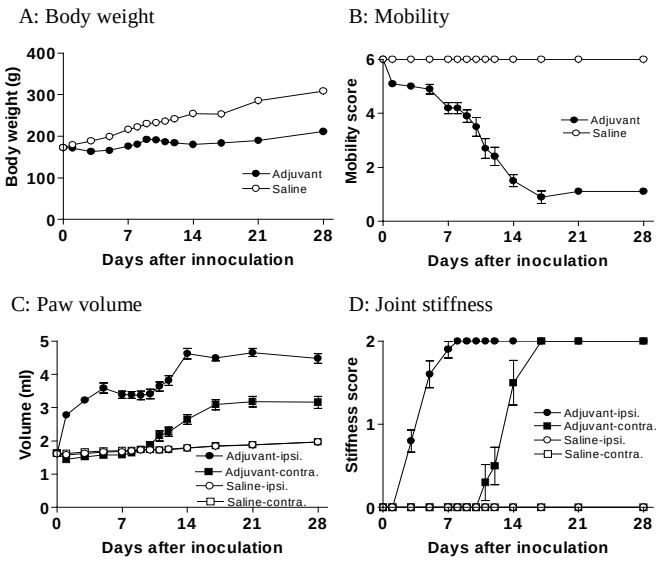

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Figure 3

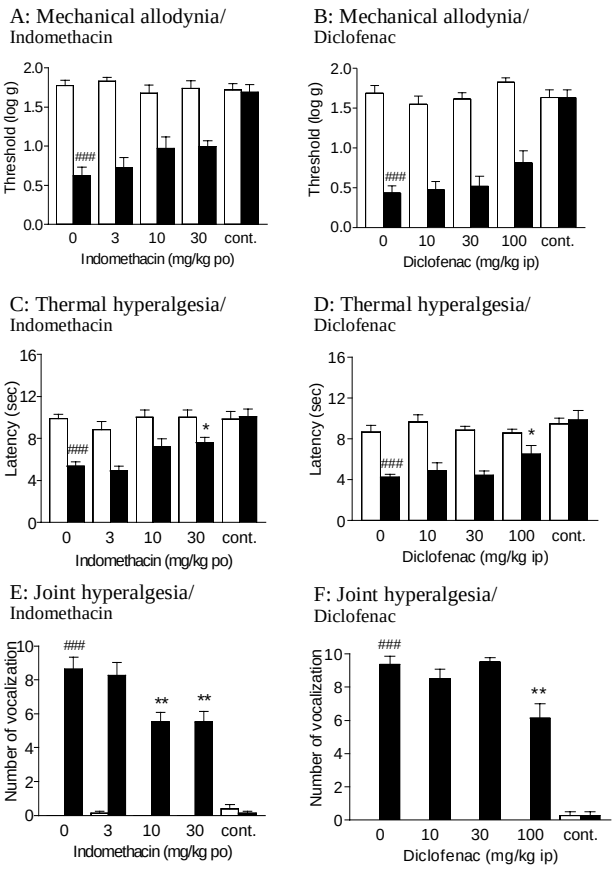

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Figure 4

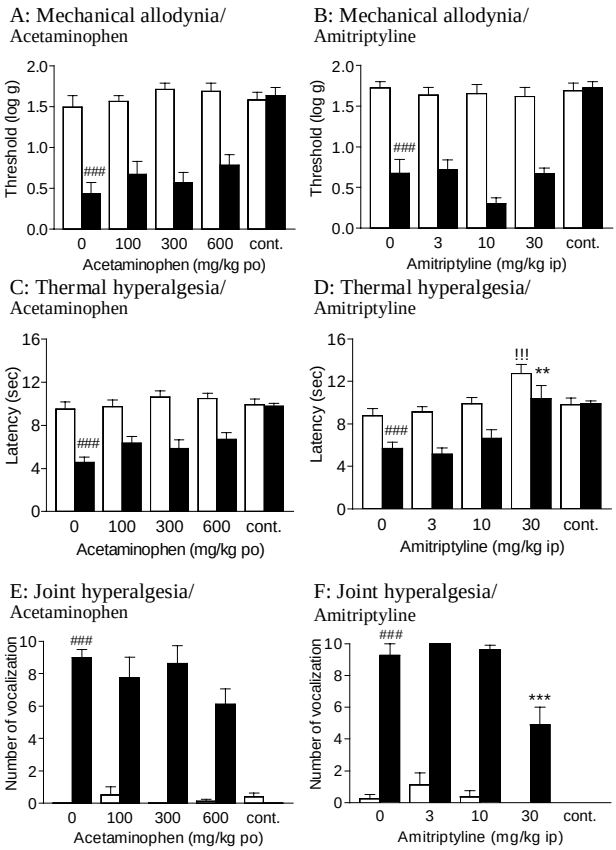

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Figure 5

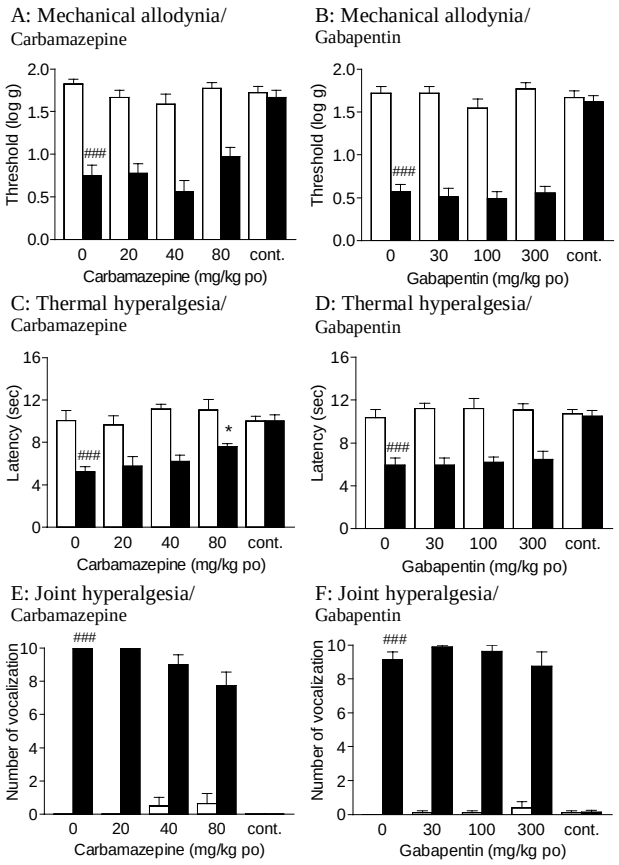

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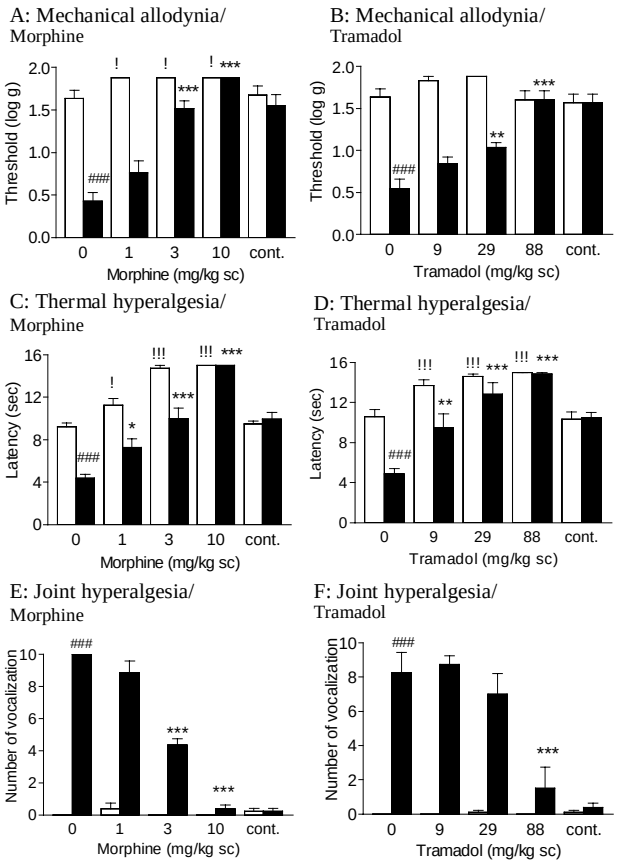
Figure 6



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