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Unilateral Inflammation of the Hindpaw in Rats as a Model of Prolonged Noxious Stimulation: Alterations in Behavior and Nociceptive Thresholds

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STEIN, C., M. J. MILLAN AND A. HERZ. *Unilateral inflammation of the hindpaw in rats as a model of prolonged noxious stimulation: Alterations in behavior and nociceptive thresholds.* PHARMACOL BIOCHEM BEHAV 31(2) 445–451, 1988.—Unilateral intraplantar injection of Freund's complete adjuvant (FCA) into one hindpaw of rats led to a localized inflammation that became apparent within 12 hours and reached its peak between 2 and 3 weeks. FCA-treated rats displayed a diminished rate of body weight gain, a reduction of food and water intake and a disruption of circadian temperature regulation, as well as decreased locomotor activity and pronounced scratching behavior in the open field. Paw pressure thresholds were reduced only in inflamed paws. Contralateral, noninflamed paws showed comparable thresholds to those of control animals. Tail-flick and tail-pressure responses were not different from controls. These data suggest that FCA-treated animals experience increased noxious input from the inflamed limb and that changes in thresholds to acutely applied nociceptive stimuli are due to a peripheral hypersensitivity of inflamed tissue. The present condition resembles most closely a state of acute inflammatory pain. The term "chronic pain" in its strict sense is not appropriate in this model.

Inflammation Freund's adjuvant Nociception Open field Chronic pain Acute pain Rat

MOST animal studies on pain are concerned with the effects of acutely applied nociceptive stimulation. Chronic pain, a complex phenomenon in humans (10), is receiving growing interest by basic researchers. In this regard, one of the most widely used animal models is adjuvant-induced polyarthritis in the rat [for review, see (5,20)]. However, in addition to producing indices for pain and hyperalgesia (3, 5, 22), this treatment results in a whole-animal disease which is thought to be a T-cell mediated delayed-type hypersensitivity reaction (27,32). Following a primary inflammation at the site of inoculation, the animals develop inflammation in multiple joints and later on in eyes, ears, nose and penis. There is lymph node enlargement, impairment of liver metabolism and changes in serum chemistry. These generalized alterations make it extremely difficult to attribute the observed functional, biochemical and morphological changes in the nervous system exclusively to the increased nociceptive input presumed to occur in these animals. Thus it is highly desirable to develop alternative models that do not entail such severe systemic changes and consequently are preferable both on scientific and ethical grounds.

This study examines alterations in clinical features, behavior and responses to various nociceptive measures in a model of unilateral, localized inflammation of a hindlimb induced by Freund's adjuvant.

METHOD

Subjects

Experiments were carried out in male Wistar rats (Ivanovas, FRG) weighing 200 to 250 g at the time of inoculation. They were housed individually in cages lined with sawdust to minimize discomfort. Standard laboratory rodent chow and water were available ad lib. Room temperature and relative humidity were maintained at 22±0.5°C and 60%, respectively. A 12 hr/12 hr (8 a.m./8 p.m.) light-dark cycle was used. Unless otherwise stated, all testing was conducted in the light phase, employing separate groups of animals. The

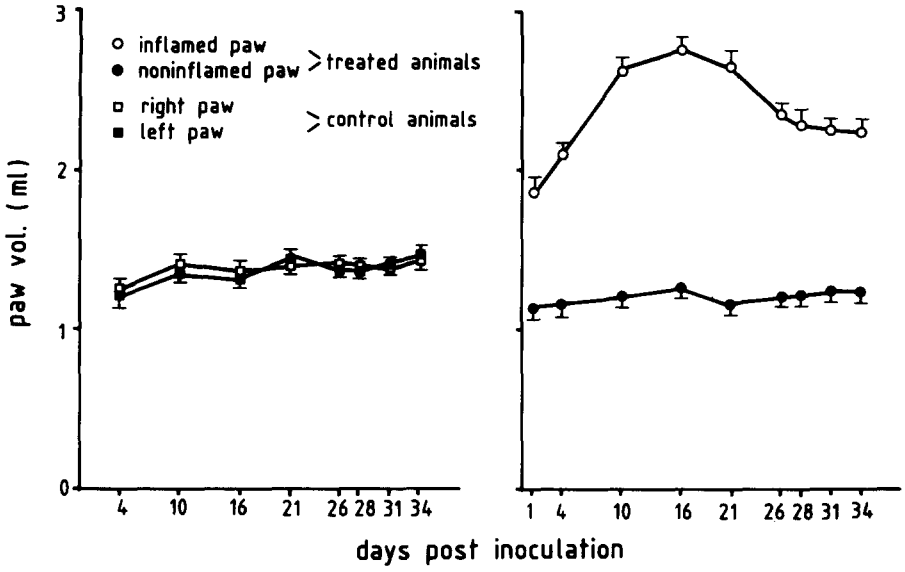

FIG. 1. Paw volumes of control animals (left) (n=10) and animals treated with intraplantar Freund's complete adjuvant (FCA) (right) (n=14). Each symbol represents the mean±standard error of the mean (SEM).

guidelines on ethical standards for investigations of experimental pain in animals (6) were followed.

Induction of Inflammation

The inflammatory agent used was modified Freund's complete adjuvant (FCA), containing 0.1% heat-killed and dried Mycobacterium butyricum in 85% Marcol 52 and 15% Aracel A mannide monooleate emulsifier (Calbiochem, La Jolla, CA). Rats received an intraplantar injection of 0.15 ml of this suspension into the right hind foot (25,27) under ether anesthesia. Control animals were anesthetized but not injected. Paw volume measurements were carried out repeatedly throughout the experiment in the following manner: Rats were gently restrained in paper wadding and the hindpaw was submerged to the tibiotarsal joint into the water filled Perspex cell of a plethysmometer (Ugo Basile, Comerio, VA, Italy). The volume of displacement, which is equal to the paw volume, was then read on a digital display.

Behavioral Characteristics

Body weight was monitored daily at 8 a.m. during the first two weeks after inoculation, at two-day intervals until day 24 and once again at 32 days postinoculation.

The quantity of food and water (available from leakproof bottles) consumed was measured at 12 hr intervals (8 a.m. and 8 p.m.) on days 1 through 12 and again on days 23 and 31. These data were pooled for analysis of the ratio of dark:light phase intake. Otherwise, measurements were taken at two-day intervals until day 24 and once again at day 32.

On days 3 (10 a.m.), 7 (10 a.m. and 10 p.m.) and 20 (10 a.m. and 10 p.m.) core temperature was measured by insertion of a digital thermistorprobe 5 cm into the rectum for 30 sec.

Open-field performance was evaluated during the dark phase (10 p.m. to 4 a.m.) of days 7, 21 and 34 in independent groups of animals. This test has been extensively described previously [for review, see (28)]. Briefly, in a dark and silent room the animal was placed into one corner of a rectangular-shaped wooden enclosure (78×96×39 cm). The field was illuminated by a red light (25 watt) located 90 cm above the floor and was divided into 20 unit squares of 19×19 cm. Each animal was observed for a period of 10 min and the following indices were recorded: Locomotor activity, defined as the number of unit squares entered by the rat during the observation period, the criterion for entry being that all four feet had to be in the square; entries into center units and perimetric units, which were recorded separately for reasons elaborated in (28); the frequency of rearing (standing on the hindpaws for a minimum duration of 2 sec) and defecation; duration of grooming and scratching. Scratching behavior, as defined in (8), entailed biting the skin, pulling the toes from between the clenched teeth or vibrating the elevated hindpaw near the ear. In the two groups tested on day 7 and 21, core temperature before and after open-field testing was also measured as described above. The field was cleaned with a water-soaked sponge prior to each observation period.

Algesiometric Testing

Three tests were used. The time course of inflammation-induced alterations in nociceptive thresholds to pressure applied to the hindpaws was monitored by administration of the first test at intervals specified in the figures. At one week postinoculation, all three tests were carried out in independent groups of animals.

Paw withdrawal to pressure. The animal was gently restrained under paper wadding and incremental pressure via a wedge-shaped, blunt piston was applied onto an area of 1.75 mm^2 of the dorsal surface of the hindpaw by means of an automated gauge (Ugo Basile, Comerio, VA, Italy). The

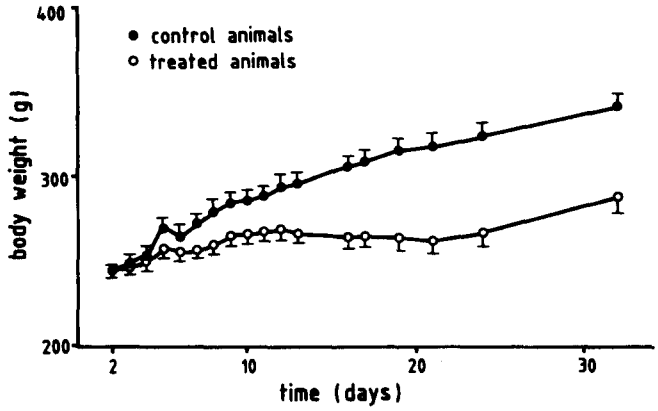

FIG. 2. Body weight of control animals (●) (n=10) and FCA-treated animals (○) (n=14). Means±SEM.

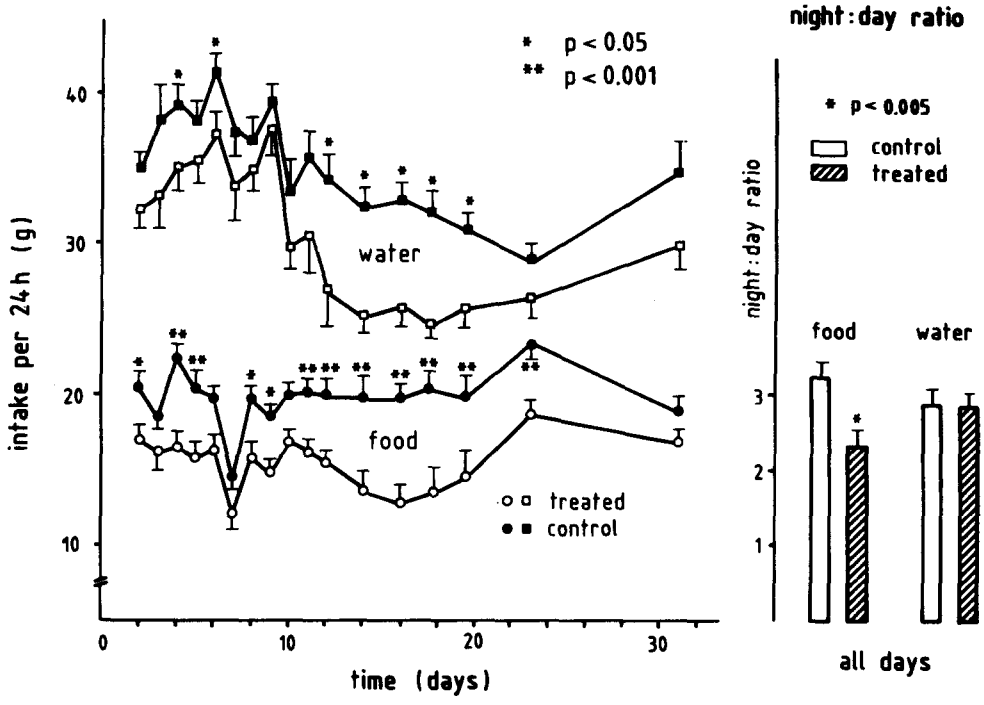

FIG. 3. Food and water intake per 24 hr. Columns depict the ratio of dark:light phase consumption. Means±SEM (Controls: n=10, treated: n=14). Asterisks indicate significance (Student's *t*-test).

pressure required to elicit paw withdrawal, the paw pressure threshold (PPT), was determined. A cut-off of 250 g was employed. Three consecutive trials, separated by intervals of 10 sec, were conducted and the average determined. The same procedure was then carried out on the contralateral side; the sequence of sides was alternated between subjects to preclude "order" effects.

Tail withdrawal to pressure. The protocol was essentially the same as described above, but with pressure applied onto the tail. The mean of three consecutive measurements at three different points of the tail (2, 3 and 4 cm from the tip) was determined. A cut-off of 500 g was used.

Tail-flick to heat. The animal was restrained under paper wadding. A focused light beam was directed at the tip of the tail and the latency of tail withdrawal was measured. A cut-off of 10 sec was imposed to prevent tissue damage. Four consecutive tail-flick latencies (separated by 10 sec intervals) were recorded and the mean of the last three was used.

Statistics

Student's two-tailed *t*-test and paired sample *t*-test were used as indicated below.

RESULTS

Inflammation and swelling of the injected paws was apparent within twelve hours of treatment with Freund's adjuvant. By 24 hr, volumes of inflamed paws were significantly higher ($p<0.05$, paired-sample *t*-test) than those of the contralateral, noninflamed paws (Fig. 1) or hindpaws of control animals ($p<0.05$, Student's two-tailed *t*-test) (Fig. 1). The inflammation increased progressively, reached a peak at 16

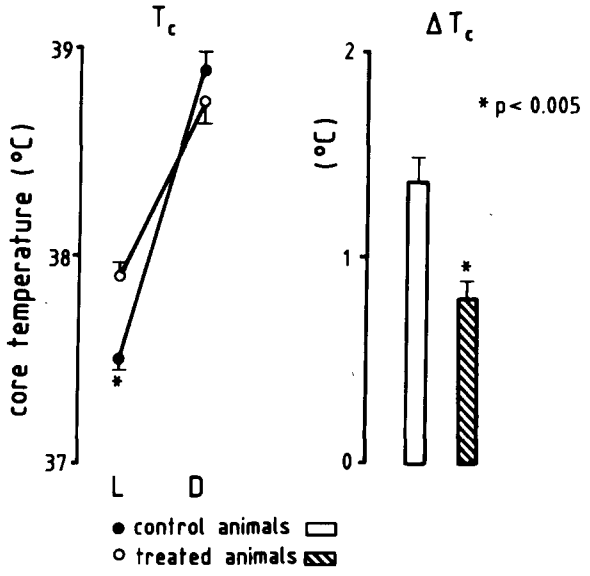

FIG. 4. Core temperature during light (L) and dark phase (D). Columns depict difference (ΔTc) between light and dark phase temperatures. Means±SEM; controls: n=10, treated: n=14. Significance indicated by asterisk (Student's *t*-test).

days and then slowly subsided. It did not disappear completely within the observation period of 5 weeks. The inflammation remained confined to the inoculated paw in all rats during the first two weeks but spread to the contralateral hindpaw in ca. 10% of the animals by the end of the experiment (34 days).

Behavioral Characteristics

Alterations in spontaneous behavior of treated animals included an obvious reluctance to place weight on the inflamed paw, limping and a persistent flexion of the knee joint on the affected side. Apart from these changes, rats responded "normally" (i.e., not aggressively or hyperreactively) to disturbance by, for example, touching or sudden noise and appeared otherwise to be in good health.

Body weight. Animals treated with Freund's adjuvant showed a diminished rate of body weight gain which was evident on day 3 postinoculation (Fig. 2). The difference between weights of treated and untreated rats became statistically significant on day 5 ($p<0.05$ at each time point, Student's two-tailed *t*-test).

Food and water intake. Adjuvant-treated rats displayed a decrease in total daily food and water intake. This decrease attained significance ($p<0.05$, Student's *t*-test) on day 2 for food and on day 4 for water intake (Fig. 3). It was most pronounced during the period of peak inflammation (days 12 through 19). The reduction of food intake occurred mainly during the dark phase of the diurnal cycle (not shown). The ratio of dark- to light-phase intake of food, but not water, was significantly lower ($p<0.005$, Student's *t*-test) in affected animals (Fig. 3).

Core temperature. Dark- and light-phase core temperature were, respectively, lower and higher in treated animals than in controls (Fig. 4). This change is reflected in the significantly ($p<0.005$, Student's *t*-test) reduced difference between dark- and light-phase temperature in treated animals (Fig. 4).

Open-field behavior. Animals treated with Freund's adjuvant displayed a pronounced reduction in locomotor activity as measured by entries into both center ($p<0.05$, Student's *t*-test) and perimetric ($p<0.005$, Student's *t*-test) squares (Fig. 5). The amount of rearing was also significantly ($p<0.001$, Student's *t*-test) less in these animals. These differences were maintained throughout the observation period (1, 3 and 5 weeks postinoculation). Defecation, as measured by the number of pellets delivered, was not markedly altered at one week, but was significantly increased in treated animals at three ($p<0.05$, Student's *t*-test) and five weeks ($p<0.005$, Student's *t*-test). Whereas the time devoted to grooming was not significantly different between treated and control groups, scratching behavior, as defined by De Castro Costa (8), occurred in treated animals exclusively (Fig. 5). The amount of scratching reached a maximum at three weeks postinoculation, concurrent with the period of peak inflammation (Fig. 5). No significant difference in core temperature elevation elicited by 10 min exposure to the open field was detected between treated and control groups (not shown).

Nociceptive Thresholds

The time course of alterations in paw pressure thresholds (PPT) is illustrated in Fig. 6. This test was only carried out through day 12 on the inflamed paw, since animals subsequently appeared exquisitely hypersensitive to manipulation of the affected limb. PPT were significantly ($p<0.05$, paired *t*-test) lower on inflamed paws than on contralateral, noninflamed paws from days 1 through 12 postinoculation. No significant differences between thresholds of left and right paws were observed in control animals (Fig. 6). Student's *t*-test revealed that PPT of noninflamed paws in treated rats were not significantly different from those in controls throughout the period of observation.

Reaction thresholds to noxious pressure or heat applied to the tail did not differ between groups (Fig. 6). These tests were carried out on days 4 and 8 postinoculation.

DISCUSSION

In distinction to polyarthritic rats, which, in addition to arthritis of multiple joints, display a severe generalized systemic disease (14, 22, 26, 32), inoculation of a single hind limb with Freund's adjuvant resulted in a localized monolateral, peripheral inflammation. The animals were otherwise in good general condition. The inflammation, as evidenced by swelling and increase in paw volume increased steadily up to a peak at 2 weeks and then subsided slowly. During the period of peak inflammatory symptoms, the animals displayed a loss of body weight and a reduction of food and water intake. Furthermore, the diurnal rhythmicity of food intake was clearly disrupted. Several factors may mediate these changes in ingestive behavior: Firstly, corticotropin-releasing factor, a potent anorexic agent (24), may be increased in these animals, as has been demonstrated in polyarthritic rats and other stress-related conditions (12). Secondly, β-endorphin, which has been shown to inhibit feeding (24), is increased in plasma of these rats (21). Thirdly, it is most likely that the motor activity which is required to obtain food causes discomfort and thus, the animals avoid such activities. Fourthly, these animals conceivably experience some distress, which, as shown in humans, is often accompanied by a depression of drives (19) and decrease in food intake.

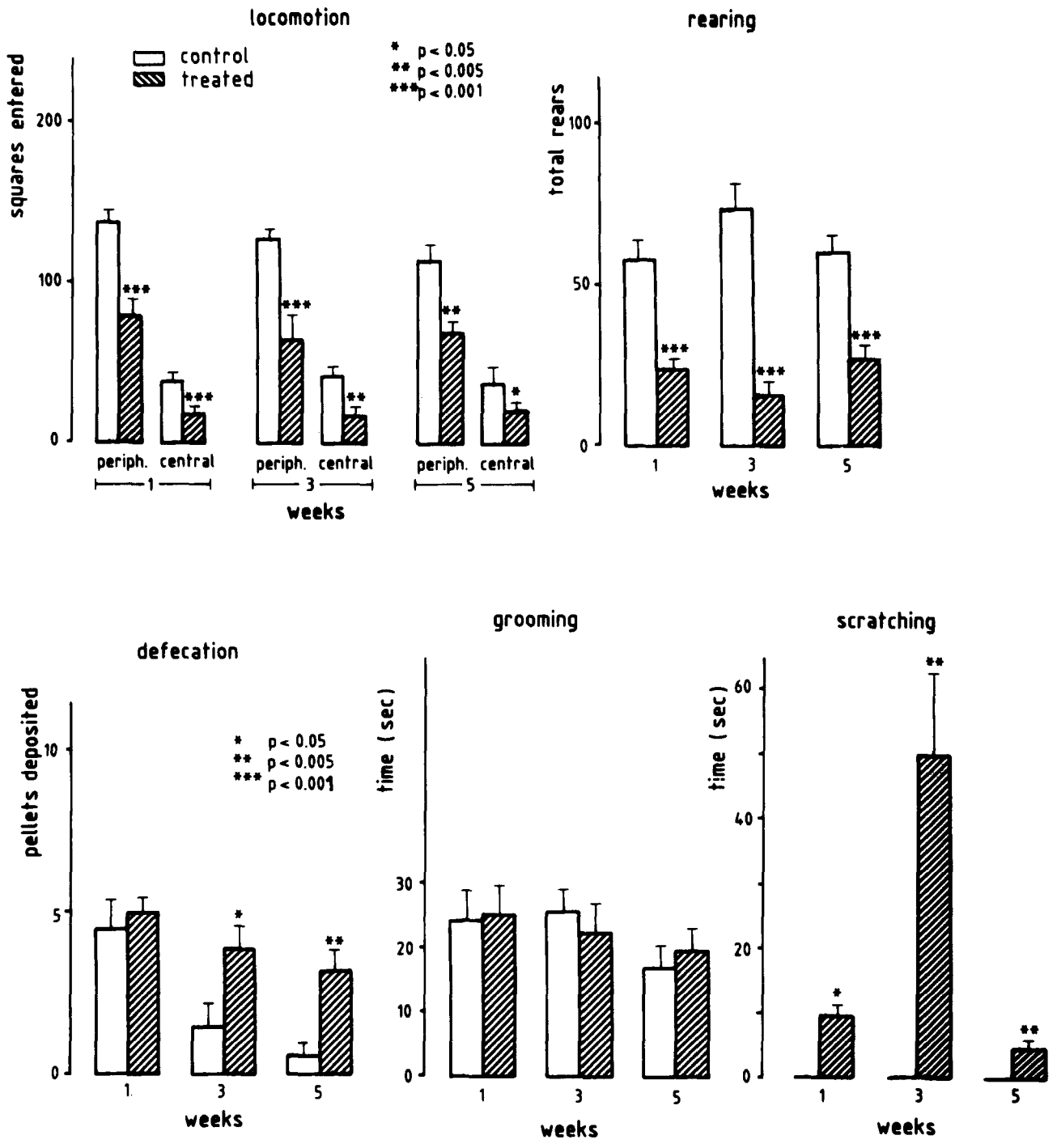

FIG. 5. Open-field behavior of control (n=10) and FCA-treated (n=14) rats at 1, 3 and 5 weeks postinoculation. Means±SEM, significance indicated by asterisks (Student's *t*-test). Detailed explanation see text.

The observed alteration in temperature regulation (i.e., the reduced difference between dark- and light-phase core temperature) may be a secondary effect of the shift in food intake. Patterns of food intake have been shown to influence circadian rhythmicity of temperature (1).

The most apparent effect of hind limb inflammation on spontaneous behavior was a sustained flexion of the knee joint and a reluctance to place weight on the affected paw. In line with this observation is the substantial decrease in locomotor activity and rearing displayed in the open-field test. These findings are similar to those obtained in polyarthritic rats (8,17) and are consistent with the view that these

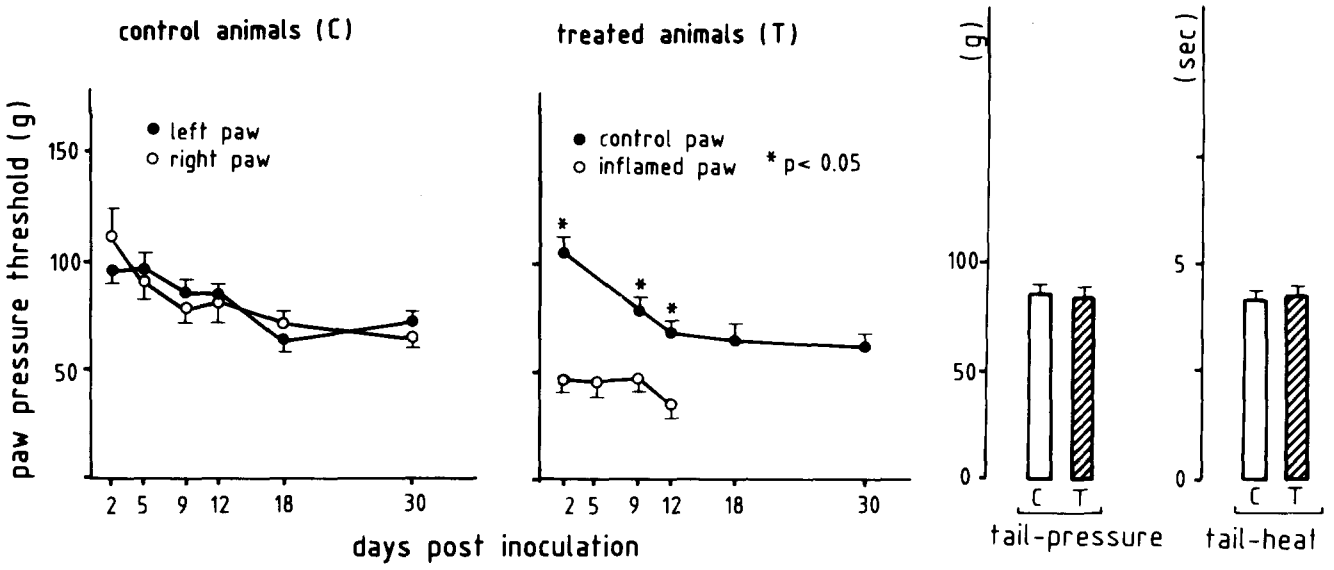

FIG. 6. Nociceptive thresholds. Time course of paw pressure thresholds of control (C) (n=10) and FCA-treated (T) (n=14) animals. Means±SEM. Significance indicated by asterisks (paired *t*-test). Columns show tail-pressure thresholds and tail-flick latencies in control (C) (n=10) and treated (T) (n=14) animals at one week postinoculation. Means±SEM.

animals experience pain upon active movement of the inflamed limb. Another indicator of pain, although controversial, is scratching behavior (2, 5, 8, 9). This occurred in treated rats only and its amount coincided with the occurrence of peak inflammatory symptoms as well as the above described behavioral changes. These results strongly support the notion that in these animals scratching is a sign of increased noxious input from the affected limb. Defecation has been suggested to represent a measure of "emotionality" or fear elicited by the open-field situation (28). Thus, the relative increase of defecation in affected animals (as compared to controls) indicates that, as the condition progresses, exposure to the open field becomes more stressful for these rats. This finding, again, may be related to increasing noxious stimulation from the inflamed limb. Open-field elicited hyperthermia has been related to stress and endorphin release (23). Our findings, however, do not suggest that, in this regard, treated animals react differently from normal rats.

Nociceptive Thresholds

Animals inoculated with Freund's adjuvant displayed a pronounced hypersensitivity to pressure applied to the inflamed paw. This finding is consistent with data obtained in polyarthritis (22) and in carrageenin-induced hindlimb inflammation (11,16). Electrophysiological studies in a similar model have demonstrated an increased responsiveness of ipsilateral nociceptive lamina I projection neurons (13) and increased spontaneous Aδ and C-fiber activity (29). It is likely that algogenic substances such as prostaglandins, bradykinin and substance P contribute to a sensitization of peripheral sensory fibers (15). It should be emphasized that thresholds to pressure were decreased only in inflamed paws and that thresholds in contralateral, noninflamed paws were the same as those in control animals. Furthermore, thresholds to noxious stimulation of the tail were not different between treated and control animals. These findings support the contention that, in this model, changes in sensitivity to nociceptive stimuli are due to a peripheral hypersensitivity of inflamed tissue and not to generalized alterations in nociceptive systems at the level of the central nervous system which have been suggested to occur in a similar condition in mice (18). In fact, we have shown that such generalized alterations, at least in regard to opioid systems, do not occur in this model (21). However, a localized increase in ir-dynorphin and ir-Met-enkephalin was demonstrated in the ipsilateral dorsal quadrant of the lumbar spinal cord of these rats (21,30). Whether this finding may provide an explanation for the observed hypersensitivity to noxious paw pressure remains a matter of speculation. Interestingly, excitatory effects of opioids have been reported recently (4,7).

Finally, the issue whether adjuvant-induced hindlimb inflammation may represent a model for chronic pain has to be addressed: Pain associated with an acute disease or traumatic injury is termed acute pain. Unlike acute pain, which proves useful in signaling injury or disease, chronic pain serves no biologic function (31). There are three types of chronic pain: (1) pain related to a chronic degenerative disease or persisting neurologic condition, (2) pain that persists beyond the normal healing time for an acute injury or disease, or (3) pain that emerges and persists without identifiable organic cause. Neither of these three definitions is applicable to the present condition. Ad 1): The time course of inflammatory symptoms in this study as well as in others (22) indicate that FCA-induced inflammation will eventually subside and resolve. Thus the present situation cannot be termed "chronic degenerative." Ad 2): There is no indication that pain will persist beyond healing time in these animals. Ad 3): All behaviors indicating that these animals experience increased nociceptive input (e.g., limping, decreased locomotor activity, hypersensitivity to acute noxious stimuli) are clearly related to obvious acute tissue pathology. Thus, the present model most closely resembles a state of acute inflammatory pain that is most likely to resolve

completely once the tissue pathology disappears. However, since pain is a subjective experience (33), which we are naturally unable to assess in an animal model, we prefer to forebear this term. Hence, we propose to designate this a condition of prolonged elevation of nociceptive input but not a model for "chronic pain."

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