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Strain Differences in Neuropathic Hyperalgesia

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LOVELL, J. A., S. L. STUESSE, W. L. R. CRUCE AND T. CRISP. *Strain differences in neuropathic hyperalgesia*. PHARMACOL BIOCHEM BEHAV **65**(1) 141–144, 2000.—The purpose of this study was to investigate strain-related differences in the onset and maintenance of thermal hyperalgesia following the induction of peripheral nerve injury in two inbred strains of rats (Fischer 344 and Lewis) and two outbred strains of rats (Sprague–Dawley and Wistar). Neuropathic pain was induced via unilateral ligation of the left sciatic nerve with chromic gut sutures. A plantar analgesia meter was used to measure paw-withdrawal latency from the ligated vs. unligated hind paws of inbred vs. outbred strains of rats to investigate strain-related differences in nerve injury-induced thermal hyperalgesia. The results demonstrated no significant effects of animal strain on presurgical paw-withdrawal latency values. Following the sciatic nerve ligation (SNL) surgery, a significant hyperalgesic response was elicited from the Sprague–Dawley and Wistar rats (outbred strains) for at least 28 days. Conversely, data analyses from the inbred strains failed to demonstrate significant hyperalgesic responses to peripheral nerve injury, with the exception of postsurgical day 10. These data emphasize the importance of considering the strain of the rat being investigated before extrapolating the results from animals experiments to treatment strategies for humans with chronic neuropathic pain.

Neuropathic pain Hyperalgesia Rat strain Chronic pain

NEUROPATHIC pain is broadly defined as pain arising from nerve injury, and some of the more common clinical peripheral neuropathies include postherpetic neuralgia (shingles), reflex sympathetic dystrophy, diabetic neuropathy, and phantom limb pain. One of the most prominent symptoms of chronic nerve pain is hyperalgesia, which is defined as an enhanced response to a normally painful stimulus. Although the pathophysiological mechanisms underlying chronic pain of neuropathic origin are not completely understood, it is clear that hyperalgesia is triggered by enhanced neuronal excitability of peripheral Aδ and C fiber primary afferent nociceptors, as well as hyperexcitability of nociresponsive neurons in the spinal cord (11).

Neuropathic pain syndromes indiscriminately cross age and gender boundaries, and are often unresponsive to conventional analgesics (1–3). Past attempts to study the mechanisms underlying chronic neuropathic pain were hampered by the lack of suitable animal models, but a preclinical animal model has since been developed that produces hyperalgesic symptoms in rats that are similar to those reported in humans (4). It has recently become apparent that different strains of rats are often used to elucidate the pathogenesis of neuropathic pain, but little is known about strain-related differences in the responsiveness of these animals to chronic peripheral neuropathies. The purpose of the present study was to investigate strain-related differences in the thermal hyperalgesic response to peripheral nerve injury.

The fact that various rodent strains have been used to investigate differences in pain sensitivity and treatment can be confusing when attempts are made to extrapolate these preclinical findings to clinical applicability. A number of reports describing strain-related differences in the antinociceptive efficacy of pharmacological agents have illuminated some interesting points. For instance, the magnitude and duration of morphine-induced analgesia is amplified by restraint stress, and outbred strains of rats exhibit a more robust stress-induced response to morphine than do inbred strains (12). Moreover, notable differences in the behavioral sensitivity to the cholinergic receptor agonist oxotremorine have been reported between separate inbred strains of mice (10). Because different strains of rats are used to study the effects of aging and/or gender on the perception and treatment of peripheral neuropathies (6–8), it seems essential to clarify how the symptomatology of chronic neuropathic pain may change as a func-

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tion of rat strain. These findings will have broad implications for interpreting the results of preclinical investigations, and for improving patient care by developing more effective treatment strategies for patients with chronic neuropathic pain disorders.

METHOD

Animals

All experiments were fully approved for the humane use of animals by the Institutional Animal Care and Use Committee at Northeastern Ohio Universities College of Medicine. Strain-related differences in the onset and maintenance of thermal hyperalgesia were evaluated in 12–16 week old male inbred strains (Fischer 344 and Lewis rats) and outbred strains (Sprague–Dawley and Wistar rats). By definition, inbred strains are produced by mating 20 or more consecutive generations of brothers and sisters (5). Inbred animals generally have greater than 98% genetic identity between individuals while retaining a very small degree of genetic diversity. On the other hand, outbred stocks are bred to maintain maximum genetic heterogeneity. An outbred stock is noninbred by purposely using a system of mating to make inbreeding virtually nonexistent (Charles River Product Catalog, 1997).

In the present studies, all rats were individually housed in clear plastic cages (19.0 × 10.5 × 8.5 inches) containing at least 4 cm of shredded aspen bedding covering the floor of the cage.

Sciatic Nerve Ligation (SNL) Surgery

The method of Bennett and Xie (4) was followed for unilateral placement of 4.0 chromic gut ligatures around the left sciatic nerve of rats (n = 6 rats/strain). Prior to the sciatic nerve ligation (SNL) surgery, rats were weighed and treated with pentobarbital 45 mg/kg, IP. The left sciatic nerve was exposed and, proximal to the trifurcation of the sciatic nerve, approximately 7 mm of the nerve was freed of adhering tissue. Four 4.0 chromic gut sutures were loosely tied around the left sciatic nerve at intervals of approximately 1 mm, and the ligatures were tied loosely enough so that, on visual inspection, blood flow was not obstructed. The right sciatic nerve was separated from the adhering tissue and muscle, but the chronic gut sutures were not tied around the nerve itself because it served as the control (sham-ligated) condition. The surgical incisions on both hind limbs were sutured and postsurgical recuperation was monitored daily. Animals were also weighed on postsurgical days 2, 5, 7, 10, 14, 21, and 28 to determine if peripheral nerve injury caused significant weight loss in the various strains of rats.

Analgesiometric Assay

A plantar analgesic meter (Model 7370; Ugo Basile, Varese, Italy) was used to measure paw withdrawal latencies in response to a thermal nociceptive stimulus applied to the ligated and unligated hind paw of rats. Paw withdrawal latency (PWL) was defined as the time from the initial exposure of the thermal stimulus to the time of withdrawal of either the ligated or unligated the hind paw from the radiant heat source. These values were recorded to the nearest 0.1 s. A 20-s cut-off limit was imposed to avoid damaging paw tissue. Two days prior to the SNL surgery, animals were individually placed in one of three separate Plexiglas containers to obtain presurgical PWL values. Following a 15-min acclimation period, a heat source was applied to the left (ligated) hind paw, followed by the right (control) hind paw 5 min later. The heat source was adjusted to generate presurgical baseline PWL values between 4–6 s. Three PWL values were taken, and the last two were averaged to obtain a presurgical mean.

To detect differences in the onset and duration of SNL-induced thermal hyperalgesia in the inbred versus outbred animals, PWL values were obtained from the ligated and unligated hind paws of each rat on postsurgical days 2, 5, 7, 10, 14, 21, and 28. Three postsurgical PWL scores were recorded on each testing day, and the last two values were averaged. To determine the extent to which thermal hyperalgesia developed from the SNL surgery, difference scores were computed for each rat by subtracting the PWL of the unligated hind paw from the PWL of the ligated hind paw. In this manner, hyperalgesia was manifested as a negative difference score. Presurgical PWL values were statistically analyzed with a one-way repeated ANOVA to determine if significant strain-related differences existed prior to the SNL surgery. A two-way repeated measures ANOVA was used to detect postsurgical differences in thermal hyperalgesia as a function of rat strain. Subsequent contrast effects analyses were used to determine if significant strain-related differences could be detected across testing days.

RESULTS

Presurgical PWL values from the left and right hind paws did not differ significantly as a function of strain ($p < 0.148$). Depicted in Fig. 1 is the time course for the onset and duration of thermal hyperalgesia following surgical ligation of the left sciatic nerve in Fischer 344 and Lewis rats (inbred strains). At no time during the 28 day postsurgical testing pe-

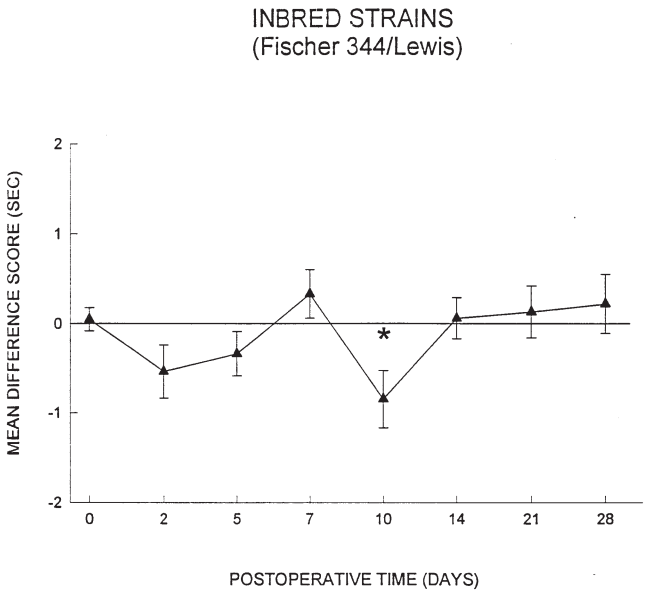

FIG. 1. Time course for the onset and maintenance of thermal hyperalgesia following the SNL surgery in inbred rat strains. Data represent difference scores ± SEM from n = 6 Fischer 344 and six Lewis rats. On postsurgical day 10, a short-lasting but significant thermal hyperalgesic response was elicited by the inbred strains. (*$p < 0.05$ compared to presurgical baseline values.)

TABLE 1

DIFFERENCE SCORES OBTAINED FROM THE INBRED STRAINS

Rat Strain (Inbred)	Day Post-SNL Surgery	Mean D.S. ± (SEM)
Fischer 344	0 (Pre-SNL)	0.06 (0.19)
($n = 6$)	2	−0.39 (0.47)
	5	−0.22 (0.48)
	7	0.56 (0.35)
	10	−0.80 (0.55)
	14	0.17 (0.39)
	21	0.36 (0.51)
	28	0.90 (0.35)
Lewis	0 (Pre-SNL)	0.02 (0.19)
($n = 6$)	2	−0.69 (0.40)
	5	−0.23 (0.74)
	7	−0.02 (0.44)
	10	−1.00 (0.33)
	14	−0.05 (0.29)
	21	−0.10 (0.30)
	28	−0.55 (0.39)

TABLE 2

DIFFERENCE SCORES OBTAINED FROM THE OUTBRED RAT STRAINS

Rat Strain (Outbred)	Post-surgical Day	Mean ± (SEM)
Sprague–Dawley	0 (Pre-SNL)	0.28 (0.27)
($n = 6$)	2	−0.24 (0.32)
	5	−0.74 (0.29)
	7	−0.73 (0.42)
	10	−0.99 (0.33)
	14	−0.62 (0.55)
	21	−0.58 (0.53)
	28	−0.49 (0.69)
Wistar	0 (Pre-SNL)	0.47 (0.25)
($n = 6$)	2	−1.03 (0.38)
	5	−0.47 (0.46)
	7	−0.71 (0.44)
	10	−0.85 (0.38)
	14	−0.17 (0.46)
	21	−0.49 (0.42)
	28	−0.83 (0.39)

riod did the Fischer and Lewis strains differ significantly from each other ($p = 0.41$). Moreover, with the exception of postsurgical day 10, the inbred animals failed to respond to the nerve ligation with significant thermal hyperalgesic responses (Fig. 1; Table 1). On postligation day 10, the Fischer 344 and Lewis strains elicited hyperalgesic responses that differed significantly from presurgical PWL values ($p < 0.05$). However, by postsurgical day 14, difference scores had returned to presurgical values and remained there for the duration of the experiment (e.g., no further hyperalgesia was noted).

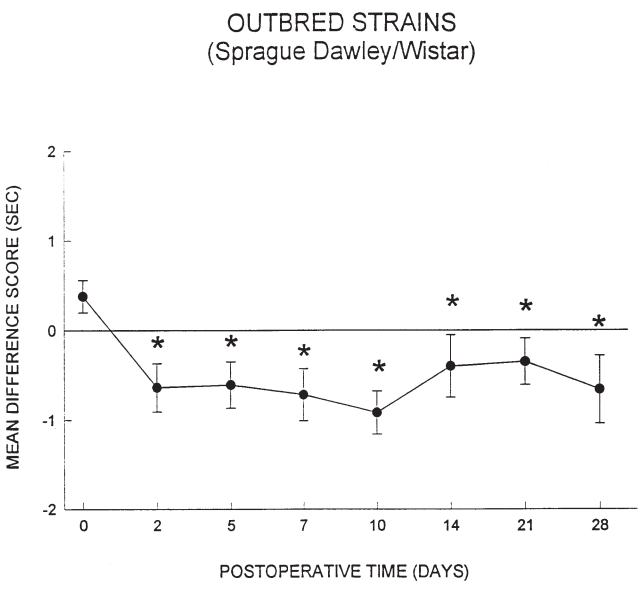

FIG. 2. Time course for the development and maintenance of thermal hyperalgesia following the SNL surgery in outbred rat strains. Data represent difference scores ± SEM from $n = 6$ Sprague–Dawley and six Wistar rats. (*$p < 0.05$ compared to presurgical baseline values.)

On the other hand, ligating the sciatic nerve in the outbred strains produced consistent and long-lasting thermal hyperalgesic responses ($p < 0.05$; Fig. 2). Significant strain-related differences ($p < 0.022$) in both the onset and duration of SNL-induced thermal hyperalgesia were observed in the Sprague–Dawley and Wistar rats. The onset of thermal hyperalgesia occurred by postoperative day 2, and difference scores remained significantly less than presurgical baseline values throughout the 28-day period (Table 2). As was true for the inbred strains, the difference scores from Sprague–Dawley and Wistar rats did not differ significantly from each other throughout the entire 28-day testing period.

To determine if peripheral nerve injury caused significant weight loss, each rat was weighed prior to the SNL surgery and throughout the 28 day postsurgical period. Analysis of the data revealed no significant differences in weight between the four rat strains, before or after the SNL surgery. In fact, the weight of the rats in each strain remained relatively stable throughout the 28-day postsurgical period.

DISCUSSION

The present study was designed to assess strain-related differences in the thermal hyperalgesic response to peripheral nerve injury in rats. The results demonstrated that the outbred strains of rats were more responsive to ligating the sciatic nerve than were the inbred strains. The thermal hyperalgesic responses of the Sprague–Dawley and Wistar rats were evident by postsurgical day 2, and the effects lasted for at least 28 days. On the other hand, the Fischer 344 and Lewis rats did not develop significant hyperalgesic responses to peripheral nerve injury until postsurgical day 10, and the behavioral effects were short-lived. It should be noted, however, that even though the outbred strains exhibited statistically significant hyperalgesic responses to surgical ligation of the left sciatic nerve, the postsurgical difference scores were less than substantial (≤1 s). In more recent studies in this laboratory, we discovered that by adjusting the calibration on the plantar analgesic meter (to decrease the intensity of the heat source), we

were able to detect longer baseline PWL values as well as more robust postligation difference scores. Had the calibration on the analgesiometric assay been adjusted accordingly for the present series of investigations, it is possible that strain-related differences would have been more pronounced. Despite these methodological concerns, the finding that strain-dependent differences exist in the onset and maintenance of the hyperalgesic symptoms of peripheral neuropathies emphasizes the need to exercise caution when extrapolating data from animal studies to humans.

The mechanisms underlying strain differences in behavioral hypersensitivity to thermal nociceptive stimuli are less than clear. It is possible that strain-related alterations in proinflammatory cytokine release from macrophages near the site of nerve injury affect the magnitude and duration of neuropathic hyperalgesia. It is known, for instance, that cytokines disrupt the structural integrity of nerves (9). In C57GL/WLD mice, there is a genetic defect in the process of axonal degeneration normally evoked by the recruitment of macrophages to the site of injury. Interestingly, the hyperalgesic response of C57GL/WLD mice to the SNL surgery is significantly attenuated when compared to normal mice (9). Thus, whereas little is known about the mediatory role of cytokines in the behavioral manifestations of neuropathic hyperalgesia, it seems plausible that the severity of nerve pain could be influenced by hematogeneous macrophages (and subsequent cytokine release) near the site of nerve injury.

Increasing evidence suggests that peripheral nerve injury causes permanent neuroplastic changes in the central nervous system (3, 11). It is conceivable that differences in the magnitude of neuropathic pain symptoms are attributable to strain-specific neurochemical and morphological rearrangements in the spinal cord. Although speculative, the chronic nature of nerve pain may cause alterations in the neurochemical makeup of endogenous pain-inhibitory systems more so in some strains than in others. Further research is warranted to investigate strain-related differences in the regulatory dynamics of pain-modulatory systems. Until more is known about the mechanisms underlying chronic pain disorders, it is essential to recognize and define the potential contributions of the strains of animals being tested before the results derived from these studies can have clinical applicability.

REFERENCES

1. Arner, S.; Meyerson, B. A.: Lack of analgesic effect of opioids on neuropathic and idiopathic forms of pain. Pain 33:11–23; 1988.
2. Backonja, M.; Arndt, G.; Gombar, K. A.; Check, B.; Zimmermann, M.: Response of chronic neuropathic pain syndromes to ketamine: A preliminary study. Pain 56:51–57; 1994.
3. Bennett, G. J.: Animal models of neuropathic pain. In: Gebhart, G. F.; Hammond, D. L.; Jensen, T. S., eds. Proceedings of the 7th world congress on pain; Progress in pain research and management, vol. 2. Seattle: IASP Press; 1994;495–510.
4. Bennett, G. J.; Xie, Y.-K.: A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain 33:87–107; 1988.
5. Harris, R. A.; Crabbe, J. C.: Genetic animal models: A user's guide. In: Boulton, A.; Baker, G.; Wu, P. H.; eds. Neuromethods, animal models of drug addiction, vol. 24. New York: Humana Press; 1992.
6. King, M. A.; Gordon, T. L.; Crisp, T.: The treatment of chronic nerve injury pain with excitatory amino acid antagonists and a nitric oxide synthase inhibitor in young, mature and aged male rats. Soc. Neurosci. Abstr. 21:1588; 1995.
7. Mao, J.; Price, D. D.; Hayes, R. L.; Lu, J.; Mayer, D. J.: Differential roles of NMDA and non-NMDA receptor activation in induction and maintenance of thermal hyperalgesia in rats with painful peripheral mononeuropathy. Brain Res. 598:271–278; 1992.
8. Maves, T. J.; Pechman, P. S.; Gebhart, G. F.; Meller, S. T.: Possible chemical contribution from chronic gut sutures produces disorders of pain sensation like those seen in man. Pain 54:57–69; 1993.
9. Myers, R. R.; Heckman, H. M.; Rodriguez, M.: Reduced hyperalgesia in nerve-injured WLD mice: Relationship to nerve fiber phagocytosis, axonal degeneration, and regeneration in normal mice. Exp. Neurol. 141:94–101; 1996.
10. Pavone, F.; Consorti, D.; Fagioli, S.: Development differences of antinociceptive effects of oxotremorine in two inbred strains of mice. Dev. Brain. Res. 49:156–160; 1989.
11. Woolf, C. J.; Thompson, W. N.: The induction and maintenance of central sensitization is dependent on *N*-methyl-D-aspartic acid receptor activation; Implications for the treatment of post-injury pain hypersensitivity states. Pain 44:293–299; 1991.
12. Woolfolk, D. R.; Holtzman, S. G.: Rat strain differences in the potentiation of morphine-induced analgesia by stress. Pharmacol. Biochem. Behav. 51:699–703; 1995.