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Differences between the Lewis and Sprague–Dawley rats in chronic inflammation induced norepinephrine sensitivity of cutaneous C-fiber nociceptors

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Abstract

We investigated whether there are any differences between the Lewis and Sprague–Dawley (SD) rats in chronic inflammation-induced norepinephrine (NE) sensitivity of nociceptors. Activities of C-fiber nociceptors innervating rat hairy hindpaw skin were recorded in an in vitro skin-nerve preparation. Sixty-five percent of C-fibers from inflamed Lewis rats were excited by NE (10 μM), against only 38% of C-fibers from inflamed SD rats. The average of the total impulses evoked in response to NE was also significantly higher in Lewis rats. The α2-adrenoceptor antagonist CH 38083 (10 μM) and yohimbine (10 μM) consistently blocked the NE-excitation of both strains. These results show that after chronic inflammation, C-fiber nociceptors of Lewis strain rats have a stronger sensitivity to NE, and that α2-adrenoceptors are predominately involved in the NE-sensitivity of inflamed rats in both strains.

Keywords: Sympathetically maintained pain; Adjuvant arthritis; Chronic inflammation; Norepinephrine; In vitro preparation

There are several lines of evidence that the sympathetic nervous system plays a role in the maintenance of painful states. Capsaicin-evoked heat hyperalgesia of the human skin persisted longer after administration of exogenous norepinephrine (NE) [6] and capsaicin-evoked mechanical allodynia was relieved by α-adrenergic blockade [11]. Sympathetically maintained pain is commonly associated with burning sensations, which suggests the symptoms are primarily due to the activity of C-fiber nociceptors. Electrophysiological evidence supports the supposition that a subpopulation of C-fiber develops a novel excitatory response to sympathetic efferent activity or close arterial injection of NE after peripheral nerve injury [15], after sympathectomy [2], and during inflammation [7,16]. However, these observations were obtained from experiments that used different animal species. One recent report showed that there are differences in adrenergic sensitivity of neuropathic pain behavior among different strains of rats [8]. It is not yet clear, however, whether such differences are due to the differences in the sensitivity of nociceptors to NE. Moreover, there are also discrepancies in reports regarding the receptor subtype mediating adrenergic sensitivity. For example, Sato et al. [16] reported the α2-adrenoceptor mediated adrenergic sensitivity of chronically inflamed rats, while the α1-adrenoceptor was reported to be involved in adrenergic sensitivity in the same strain of neuropathic rats [9]. Therefore, we decided to reevaluate the NE-responsiveness of experimentally induced chronic inflammation, focusing on its species dependence. We studied the NE-excitability of cutaneous C-fiber from Sprague–Dawley (SD) and Lewis strains during chronic inflammation by in vitro recording [14], using a set up which allowed the concentration of drugs at the receptive field to be controlled with no influence on blood contents.

Experiments were carried out on 42 (ten untreated) outbred strain SD (SLC, Japan) and 33 (six untreated) inbred strain Lewis (Charles Rivers, Japan) rats weighing 180–250 g. Chronic inflammation was induced by intradermal injection (0.1 ml) of complete Freund's adjuvant (CFA) (a suspension of killed Mycobacterium butyricum in mineral oil- 6 mg/ml for Lewis and 12–24 mg/ml for SD) in the distal third of the tail. Signs of inflammation, e.g. swelling of the ankle joint, plantar erythema and decreased mobility appeared 2–3 weeks after treatment. The skin-nerve in vitro recording procedure has been described else-

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where [14]. In this study, units that showed a slowly adapting response to mechanical stimulation and conduction velocities <1.5 m/s, and responded either to heat or bradykinin, were considered to be the C-fiber nociceptors. NE (10 μM) was superfused into the stimulation chamber (receptive field of a C-fiber nociceptor) for 5 min at 30-min intervals and antagonists were applied 2–3 min before the first application of NE and continued during NE-application. NE-induced C-fiber discharges were weak and had low frequencies, as has been reported previously, which led us to define 'responded by NE' as at least 2 impulses/min. Spontaneous activity during the 60 s pre-stimulus period was multiplied by five and then subtracted from the 5 min count (total impulses) obtained during the stimulation period. Data was presented as mean ± SEM. Statistical analysis involved χ^2-test, unpaired *t*-test and unpaired *t*-test with Welch's correction. All experimental procedures were approved by the Animal Care Committee, Research Institute of Environmental Medicine, Nagoya University.

A total of 72 C-fibers were studied. We noted from other studies of our laboratory that in this preparation NE is effective at 10 μM, which is also the threshold concentration of dorsal root ganglion (DRG) somata of C-fiber [19]. None of the 15 C-fibers from the untreated rats of either strain responded to NE at this concentration (Table 1). On the other hand, 17 C-fibers (65%) from inflamed Lewis rats and 12 C-fibers (38%) from inflamed SD rats responded (Fig. 1A). This difference in the incidence of NE excitation between these rat strains was statistically significant ($P < 0.05$, χ^2-test). The average of the total impulses evoked by individual NE responsive fibers was also significantly higher in C-fibers from inflamed Lewis rats compared to those from inflamed SD rats (Fig. 1B; $P < 0.05$, unpaired *t*-test with Welch's correction). Twelve C-fibers (46%) from inflamed Lewis rats and ten (32%) from inflamed SD rats showed spontaneous activity at 5.1 ± 0.8 and 7.9 ± 4.6 impulses/min, respectively. Of these spontaneously discharging C-fibers, ten (83%) from Lewis and six (60%) from SD responded to NE (Table 1). The average of the total impulses evoked per NE-stimulation tended to be higher in the spontaneously discharging C-fibers, and in the case of Lewis strain the difference was significant (134 vs. 33 impulses; $P < 0.02$, unpaired *t*-test).

Two distinct qualitative patterns of NE-excitation of C-fibers were observed irrespective of strain: short burst of discharges and enhancement of the ongoing discharges. Enhancement of the ongoing discharges (Fig. 2A) was the most common pattern, and has been observed in many studies [4,10,19]. C-fibers without any spontaneous activities responded with poor impulse generation and sometimes produced a burst of discharges (Fig. 2B). Nine NE-responsive C-fibers classified with this pattern: five from Lewis and four from SD rats. Bursting discharges of C-fiber to NE was also reported in a previous study [15]. NE-induced enhancement and burst were observed at a mean latency of 86 ± 25 and 194 ± 17 s, respectively, after arrival of the substance in the stimulation chamber.

NE (10 μM) applied twice at 30-min interval in six C-fibers: four from Lewis and two from SD rats. Fig. 3 shows that first NE-response is a little larger than the next

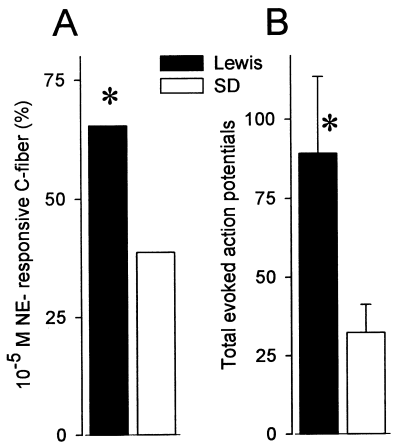

Fig. 1. NE sensitivity of cutaneous C-fiber nociceptors after chronic inflammation in the Lewis and Sprague–Dawley (SD) rats. Significant differences were evident between these strains in (A) the incidence of NE- responsive C-fiber nociceptors ($P < 0.05$, χ^2-test) and (B) average of the total impulses evoked by individual units in response to NE ($P < 0.05$, unpaired *t*-test with Welch's correction).

Table 1
Response of C-fibers to norepinephrine- superfusion[a]

	Inflamed rats		Normal rats	
	n tested	*n* excited*	*n* tested	*n* excited
Lewis	26 (12)	17 (10)	5 (1)	0 (0)
SD	31 (10)	12 (6)	10 (1)	0 (0)

[a] *See text for criteria. *n*, number of fibers; values in parentheses represent the *n* of spontaneously discharging units.

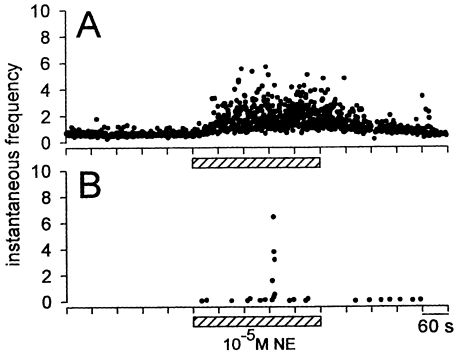

Fig. 2. Instantaneous frequency plots of the NE-response of two single C-fibers recorded from the inflamed Lewis (A) and SD (B) rats. Note the two qualitative patterns of response: enhancement of the ongoing discharges (A) and burst of discharge (B) during NE-superfusion.

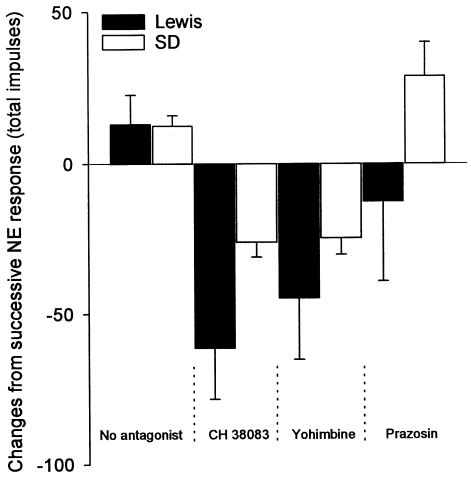

Fig. 3. Effect of the pretreatment of α-adrenoceptor antagonists on NE-induced excitation of C-fibers during chronic inflammation. Ordinate: mean ± SEM (evoked impulses during 5 min NE application) of the differences of first NE-response (in the presence or absence of antagonist) to the next. Concentration of all drugs used was 10 μM. Effect of the α2-adrenoceptor antagonists, CH 38083 and yohimbine did not differ between these strains. However, an α1 adrenoceptor antagonist, prazosin had suppressive effects in few cases of Lewis rats (see text).

(positive difference), namely, NE-response is reproducible with this application procedure.

In 23 NE (10 μM) responsive C-fibers, pretreatment of the α-adrenoceptor antagonists (10 μM) was done before the first application of NE. Based on the control study that showed reproducible NE-responses, if the second NE effect was greater than the first (≥50%), we considered that the NE-effect was blocked in the first case due to pretreatment of antagonist. In fact, in all cases, the selective α2-adrenoceptor antagonist CH 38083 (Lewis, $n = 3$; SD, $n = 3$) and yohimbine (Lewis, $n = 4$; SD, $n = 3$) completely eliminated the NE-effect of both strains. NE did not evoke any impulses in the presence of CH 38083 in all cases tested, while 0.7 ± 1.1 (Lewis) and 7 ± 7 (SD) total impulses were generated in the presence of yohimbine. Therefore, Fig. 3 shows the large negative differences from the first (in the presence of antagonist) to second NE response. Pretreatment with the α1-adrenoceptor specific antagonist prazosin had no effect on the C-fibers from SD rats (positive difference in Fig. 3; $n = 4$), but it was effective in two out of six of the Lewis rats, resulting negative difference with larger SEM. (Fig. 3).

It has been reported that α-adrenoceptor antagonists have an inhibitory effect on spontaneous activity of the afferents in neuropathic animals [9]; yet this has not been a consistent observation [4,19]. In this study, although adrenoceptor antagonists blocked the C-fiber response to NE, they did not reduce the baseline spontaneous firing rate. Rather, by themselves, CH 38083 excited 16% of C-fibers, yohimbine 28%, and prazosin 30%.

Lewis strain rats are susceptible to a wide variety of experimentally induced autoimmune and inflammatory diseases, which are considered to be due to overproduction of pro-inflammatory cytokines such as interleukin-1 and tumor necrosis factor (TNF-α) [13], while SD rats show low-grade inflammation due to decreased TNF-(mRNA expression and suppressed B cell responses [20]. In the present study, we also found that CFA-induced inflammation developed in Lewis rat with a low dose, showed a 100% incidence and was proved to be severe, consistent and stable. In contrast, 2–4 times higher concentration of CFA produced largely variable inflammatory signs in just 40% of SD rats. These strain differences in the development of inflammation might correlate with the variance in the NE-sensitivity of C-fiber from inflamed rats. Another possibility is the differences in the distribution of genetic factors associated with the adrenergic sensitivity between rats of different strains. Genetic differences were also observed in normal rats in the receptor sensitivity to catecholamine [17] and in the rate of release of catecholamine from sympathetic nerve endings to stressful stimuli [12].

In our study, we found that the α2-adrenoceptor is predominately involved in the NE-sensitivity of inflamed SD and Lewis strain rats, which is consistent with the previous reports [10,16,19]. However, an α1-adrenergic antagonist, prazosin, also suppressed the effect of NE in some inflamed Lewis rats in our study; this is contrary to the results of Sato et al. [16], who did not find a prazosin-induced suppression of the NE-effect in inflamed Lewis strain rats. Prazosin was reported to have a strong α2B antagonistic effect [3] and recently Shi et al. [18] showed that carrageenan inflammation caused a significant increase in α2B mRNA positive neurons in dorsal root ganglia of rats. Therefore, the observed suppressive effect of prazosin on C-fibers from Lewis rats may be an α2B antagonistic effect. Further study is required to determine whether the subtype of α2-adrenoceptor expression is also species or strain dependent.

The mechanisms of the inflammation-influenced appearance of NE sensitivity are yet to be determined. Increase in the number of α2A and α2C immunoreactive [1] and mRNA positive neurons [18] was found in neuropathic animals but not in inflamed animals. Instead, inflammation caused a significant increase in α2B mRNA positive neurons [18]. It remains unclear whether receptor upregulation is necessary for the emergence of functional sympathosensory coupling, since many DRG neurons of intact rats express α-adrenoceptor mRNA and protein [5]. One possibility is that this mechanism involves inactive α-adrenoceptors present in nociceptive terminals, which are activated when the excitability of the nociceptors is increased. Supporting this hypothesis is, first, that the current study shows that spontaneously discharging C-fibers were more often responsive to NE (Table 1), and second, that after bradykinin- and heat-excitation a subset of C-fibers were excited by NE in normal rats (unpublished observations). Similarly, following subcutaneous injection of a cocktail of nociceptive substances, sympathetic trunk stimulation

markedly increased C-fiber discharge [7]. From these observations it is conceivable that, following inflammation, C-fibers become sensitized by inflammatory mediators, e.g. bradykinin, prostaglandin and decreased pH, and develop novel responsiveness to NE. As the experimentally induced inflammation was more pronounced in Lewis strain rats, it would be reasonable that an increased percentage of C-fibers were sensitized and eventually responded to NE with increased magnitude in this strain.

Finally, this study provides conclusive evidence that Lewis strain rats have stronger adrenergic sensitivity, which is not particular to a single pain model. This would make them a preferable animal strain for future study to explore the unknown mechanisms of adrenergic sensitivity.

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