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Effects of Aging on the Expression of Neuropathic Pain Produced by Injury of Sciatic Nerve Branches

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Peripheral nerve injury can lead to neuropathic pain which is a chronic pain state like hyperalgesia, spontaneous pain, mechanical allodynia, and thermal allodynia. Aging may affect the severity of neuropathic pain symptoms. In humans, there is difference between old and young persons in susceptibility of neuropathic pain. The present study was conducted to determine whether the behavioral signs of neuropathic pain following peripheral nerve injury depend on age at the time of the peripheral nerve injury. Under halothane anesthesia, the tibial and sural nerves were injured and the common peroneal nerve was left intact. Neuropathic pain behaviors were compared between young and old groups of rats. Rats with injury to the tibial and sural nerves showed the vigorous mechanical allodynia, cold allodynia, and spontaneous pain. Young rat group showed more profound mechanical allodynia, cold allodynia, and spontaneous pain compared to old rat group. The results suggested that all the behavioral signs of neuropathic pain may be manifested more robustly in young rat group rather than old rat group in our animal model of neuropathic pain which was produced by injury to the tibial and sural nerves, while leaving the common peroneal nerve intact. Aging appears to affect several factors related to the generation or maintenance of neuropathic pain.

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INTRODUCTION

Abnormal pain states resulting from peripheral nerve injury are commonly referred to as neuropathic pains (Bonica, 1990; Loh and Nathan, 1978; Mitchell, 1872). This pain syndrome is usually termed neuropathic pain and includes spontaneous pain, hyperalgesia (an increased sensitivity to painful stimuli), and allodynia (the perception of normally innocuous stimuli as painful).

Recently, we developed a neuropathic pain rat model employing distal sciatic nerve branch injury (Lee, Won, Baik, Lee, & Moon, 2000). In our neuropathic pain model, the tibial and sural nerves were transected and the common peroneal nerve was left intact. The rats show vigorous ongoing spontaneous pain, mechanical allodynia, and cold allodynia after injury. These neuropathic pain behaviors were not relieved by functional sympathectomy using guanethidine (Lee et al., 2000). This indicates that our model of neuropathic pain represents sympathetically independent pain rather than sympathetically maintained pain.

There were reports indicating that the age and size of rats affect the development of allodynia in other neuropathic pain model (Chung, Choi, Yoon, & Na, 1995; Kim, Na, Yoon, Nahm, Ko, & Hong, 1995; Tanck, Kroin, McCarthy, Penn, & Ivankovich, 1992). Some studies reported that younger animals displayed more profound neuropathic pain than older animals (Chung et al., 1995; Tanck et al., 1992). In contrast, there is a report that older rats displayed more vigorous mechanical allodynia than younger rats (Kim et al., 1995). However, it is uncertain why behavioral signs of neuropathic pain are expressed differently between young and old animals. The present study was conducted to examine the effects of aging on the expression of neuropthic pain symptoms in our rat model of neuropathic pain.

MATERIALS AND METHODS

Subjects and Surgery

Two groups of male Sprague-Dawley rats were used. Young rats (n=17) weighed 170-220g (6 weeks postnataly) and old rats (n=17) weighed 450-550g (8 month postnataly). All experiments were conducted in accordance with guidelines accepted by the International Association for the Study of Pain. Under halothane anesthesia, the thighs and backs of the animals were shaved and a skin incision was made in the region of the thigh. The muscle was retracted and the three major divisions of the sciatic nerve (the tibial, sural and peroneal nerves) were clearly separated by individual perineurium. In order to produce neuropathic injury, the left tibial and sural nerves were tightly ligated using 6-0 silk thread and cut with fine scissors, while the common peroneal nerve was left intact (Lee et al., 2000). Hemostasis was confirmed and the wound was sutured. Rats were allowed to have 24 hours to recover from the surgery prior to behavioral testing.

Behavioral tests for neuropathic pain

Behavioral signs representing different components of neuropathic pain (mechanical allodynia, cold allodynia, and spontaneous pain) were examined on all the rats for ten weeks postoperatively. In order to measure mechanical allodynia, rats were placed on a metal mesh floor under a transparent plastic dome (8×8×18cm), and innocuous mechanical stimuli were applied with a von Frey filament (8mN of bending force) to the sensitive area of the left (injured) or right (intact) hind paw. The most sensitive area was first determined by poking various areas of the paw with a von Frey hair. Then, the actual test was conducted by gently poking the spot with the filament. The von Frey filament was applied 10 times (once every 3-4 sec) to each hind paw. The frequency of foot withdrawal out of 10 trials of the von Frey filament application was expressed by the percentage (response rate (%) = no. of foot withdrawals / no. of trials (10) × 100). To quantify cold sensitivity of the paw, rats were placed on the metal mesh floor under the transparent plastic dome and brisk paw withdrawal in response to acetone application was measured. Acetone was applied 5 times (once every 5 min) to each paw. The frequency of foot withdrawal out of 5 trials of acetone application was expressed by the percentage (response rate = no. of foot withdrawals / no. of trials (5) × 100). To measure spontaneous ongoing pain, each rat was placed on an acrylic plate at room temperature and covered by a transparent plastic dome. After 5 min of adaptation, the cumulative duration of time that the rat held its foot off the floor for additional 5 min was used as the ongoing pain index.

Statistical analysis

Data were expressed as mean ± s.e.m. Differences in neuropathic pain behaviors between young and old groups of rats following nerve injury were tested with a Student's t-test. Statistical significance was inferred at the $p<.05$ level.

RESULTS

Rats in which the tibial and sural nerves were injured showed well-developed neuropathic pain behaviors. Mechanical allodynia to the von Frey filament, cold allodynia to acetone, and spontaneous ongoing pain gradually developed in rats with tibial and sural nerve injury.

Mechanical allodynia: The results of the behavioral tests for mechanical sensitivity of the paw are shown in Fig. 1. The frequency of foot withdrawals to repeated mechanical stimulation was plotted for each group against time (Fig. 1). Abscissa was marked as Pre for preoperative control, and D and W for postoperative days and weeks, respectively. Before surgery, the rats from any group were rarely responsive to the von Frey filament applied to the foot. After the injury of the tibial and sural nerves, the ipsilateral (left;

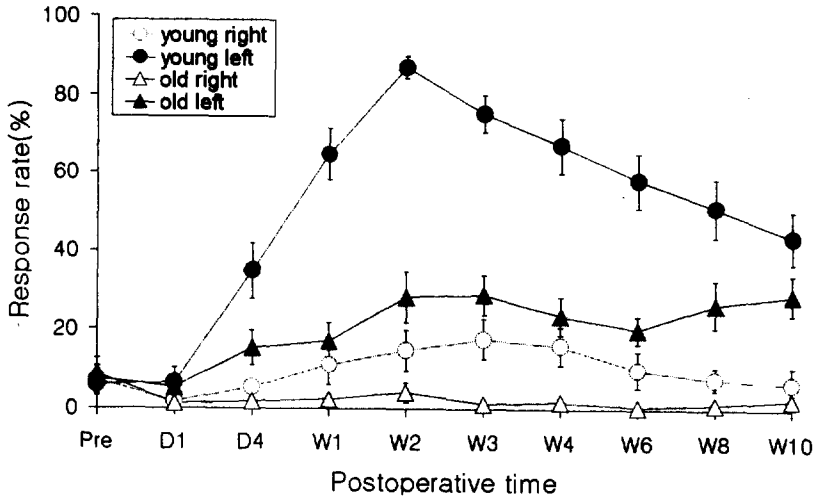

Fig. 1. Comparison of mechanical allodynia between young and old rats. Response rate to von Frey filament was used as an index of mechanical allodynia. Data are expressed as mean±s.e.m. Abscissa was marked as Pre for preoperative control, and D and W for postoperative days and weeks, respectively. Circles and triangles indicate young and old groups, respectively. Filled and open symbols indicate injured (left) and intact (right) sides, respectively. Asterisks indicate significant differences between young and old groups ($p<.05$).

injured) hind paw became sensitive to mechanical stimuli. As has been done previously, such an increase in mechanical sensitivity was interpreted as a behavioral sign of mechanical allodynia induced by peripheral nerve injury. The increase in mechanical sensitivity was much greater in the young rat group compared to the old group. In the figure, asterisks indicate significant differences between young and old rat groups. It should be noted that the contralateral (right; intact) paw commonly remained unresponsive to mechanical stimulation throughout the test period in all groups. The data suggest that neuropathic injury produces much more profound behavioral signs of mechanical allodynia in young rats.

Cold allodynia: The results of the behavioral tests for thermal sensitivity of the paw are shown in Fig. 2. The frequency of foot withdrawals to repeated cold stimulation (with acetone bubble) was plotted for each group against time (Fig. 2). Rats did not respond to thermal stimulation of the paw before neuropathic surgery. After neuropathic surgery, however, rats began to withdraw the foot when acetone was applied to the paw on the injured side (left). As has been done previously, such an increase in thermal sensitivity to acetone was interpreted as a behavioral sign of cold allodynia induced by peripheral nerve injury. The increase in thermal sensitivity was much greater in the young rat group compared to the old group.

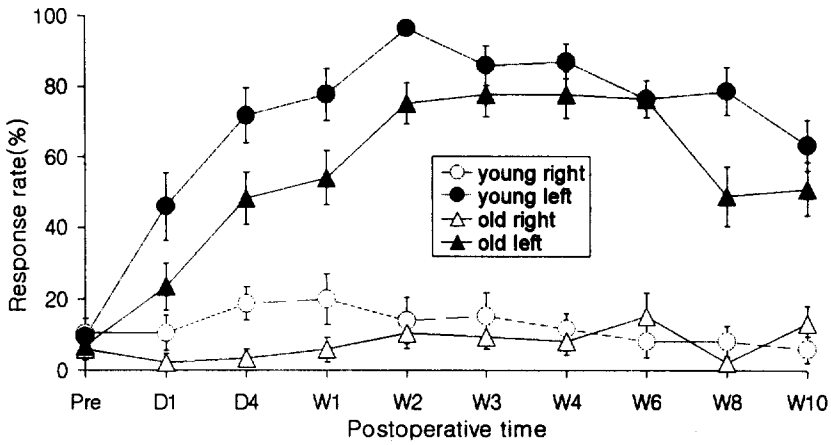

Fig. 2. Comparison of cold allodynia between young and old rats. Response rate to acetone was used as an index of cold allodynia. Details as for Fig. 1.

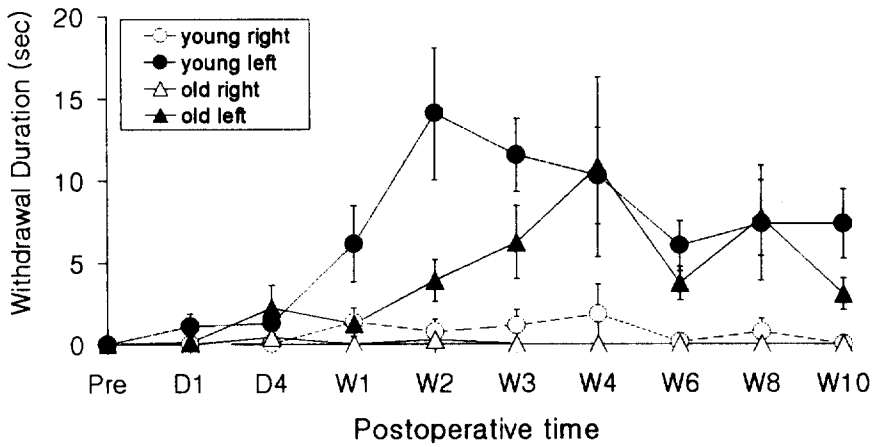

Fig. 3. Comparison of spontaneous pain between young and old rats. Cumulative duration of spontaneous withdrawal behaviors for 5 min was used as an index of spontaneous pain. Details as for Fig. 1.

In the figure, asterisks indicate significant differences from preoperative values. The contralateral (right) paw commonly remained unresponsive to acetone in all groups. The data suggest that neuropathic injury produces much more profound behavioral signs of cold allodynia in young rats.

Spontaneous pain: The results of the behavioral tests for spontaneous pain are shown in Fig. 3. The cumulative duration of time that the rat held

its foot off the floor, was plotted for each group against time (Fig. 3). Rats did not lift their injured foot spontaneously before neuropathic surgery. After neuropathic surgery, however, rats began to lift their injured foot spontaneously. As indicated in Fig. 3, the spontaneous pain was more profound in young rat group compared to old group. In the figure, asterisks indicate significant differences from preoperative values. The contralateral (intact) paw showed no spontaneous ongoing pain in all groups. The data suggest that neuropathic injury produces much more profound behavioral signs of spontaneous pain in young rats.

DISCUSSION

The results of the present study suggest that a peripheral nerve injury induces more robust neuropathic pain behaviors in young rat group compared to old rat group. These results are consistent with Tanck et al. (1992) and Chung et al. (1995) but not with Kim et al. (1995). For example, Tanck et al. (1992) reported that behavioral signs of heat hyperalgesia as well as cold and mechanical allodynia were much less pronounced in older rats, using the sciatic nerve chronic constriction model developed by Bennett and Xie (1988). Chung et al. (1995) extended Tanck et al.'s report (1992) in that the influence of age in neuropathic pain behaviors includes signs of ongoing pain. In fact, the most dramatic difference between old and young animals was found in the signs of ongoing pain. Kim et al. (1995) reported mechanical allodynia is manifested more robustly in old rats compared to young rats.

There is no clear explanation for the effect of aging on the expression of neuropathic pain behaviors at the present time. However, some postulations may at least in part explain the effects of aging observed in different studies. First, Tanck et al. (1992) proposed that the effect of aging may be due to differences in the size and structural integrity of the nerves between young and old rats. The larger, older animals failed to develop any significant allodynia to cold. The diameter of the nerves in the old rats is larger than that of the nerves in the smaller animals, with a greater amount of fibrous connective tissue surrounding the nerve. The network of epineural blood vessels was also less easily visualized because of the fibrous sheath surrounding the nerve. The increased thickness of the fibrous tissue surrounding the nerve may have prevented the development of demyelination, loss of myelinated fibers, and loss of unmyelinated fibers which have been implicated in the development of allodynia in this rat model.

Second, the difference in the animal model may differentially affect the expression of neuropathic pain. For example, Bennett and Xie's (1988) or Kim and Chung's model (1992) produces neuropathic pain on the hind paw of the rat. Na et al.'s model (Na, Han, Ko, & Hong, 1994) produces neuropathic pain on the rat tail. Neuropathic pain induced in different body sites may be affected differently by aging.

Third, it is likely due to a mechanism that involves other than anatomical differences. The

generator mechanism of ectopic discharges in the injured peripheral nerve (at the neuroma or DRG) may be influenced by aging. It is well known that ectopic discharges are produced by injured nerve fibers and their DRG cells (Han, Na, Yoon, & Chung, 1994; Kajander, Wakisaka, & Bennett, 1992). The ectopic discharges may contribute to the development of neuropathic pain and be affected by aging.

Fourth, although it is uncertain whether the degree of sprouting of sympathetic postganglionic fibers into the DRG is correlated to the degree of the neuropathic pain in a certain neuropathic pain model (Lee, Yoon, Chung, & Chung, 1998), it may be influenced by aging. Physiological or morphological changes in the intact DRGs may play a role in producing neuropathic pain. Chung et al. (Chung, Lee, Yoon, & Chung, (1996) and Lee et al. (1998) demonstrated that there is an increase in the density of tyrosine hydroxylase-immunoreactive (TH-IR) fibers as well as the number of TH-IR-wrapped neurons in the completely intact as well as injured segment in the segmental spinal nerve ligation injury model. These pathophysiological changes may be related to the production or maintenance of neuropathic pain and aging may influence either of these mechanisms. In fact, there is an evidence that sympathetic innervation of the dorsal root ganglia following peripheral nerve injury is related to aging (Ramer & Bisby, 1998).

Fifth, the effectiveness of spinal dorsal horn sensitization may be influenced by aging. Afferent signals from both intact and injured fibers would enter the spinal cord and sensitize the spinal dorsal horn neurons. The alteration of the central processing of sensory information by sensitization of the spinal cord seems to be essential for many sensory abnormalities including neuropathic pain (Campbell, Meyer, & Raja, 1992; Gracely, Lynch, & Bennett, 1992; Roberts, 1986) and this process may be affected by aging. This possibility is supported by a recent report of a decline in spinal opioid-induced antinociception as a function of age (Crisp, Stafinsky, Hoskins, Perni, Uram, & Gordon, 1994).

Sixth, collateral sprouting after nerve section may be influenced by aging. Peripheral nerve axons are capable of reinnervation to denervated areas after injury. It has been reported that there are two types of regenerative growth. One involves collateral innervation by neighboring intact fibers. For example, when the sciatic nerve is injured in rats, the saphenous nerve axons can sprout to expand their receptive field to the denervated areas (Devor, Schonfeld, Seltzer, & Wall, 1979; Kinnman & Aldskogius, 1986; Wiesenfeld-Hallin, Kinnman, & Aldskogius, 1989). The other involves regenerative outgrowth of fibers severed by the injury. After the sciatic nerve is injured, the regenerating sciatic nerve can make a functional contribution. This can be seen by the return of sensation to zones not invaded by the saphenous nerve (Devor et al., 1979). With the return of the sciatic nerve, the expanded distribution of the saphenous nerve goes back to its original boundaries. Of these two types of reinnervation, collateral sprouting seems to be involved in neuropathic pain symptoms, including allodynia and spontaneous

pain and may be affected differently between young and old rats.

These factors mentioned above appears to affect the expression of neuropathic pain differently in different aged rats. However, the specific mechanisms that aging may act on the contribution of these factors to neuropathic pain remain to be determined.

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