Changes in Cardiovascular Parameters and Plasma Norepinephrine Level in Rats after Chronic Constriction Injury on the Sciatic Nerve

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ABSTRACT

To evaluate whether neuropathic pain affects autonomic nervous activities, we investigated daily change in cardiovascular parameters and plasma norepinephrine (NE) in free-moving rats after chronic constriction injury (CCI) on the sciatic nerve. Arterial blood pressure (BP), heart rate (HR), and the power spectrum of pulse interval variability were analyzed. Daily change in motor activity and nociceptive behavior was also measured from some CCI rats. In others, NE from daily blood samples was quantified and spontaneous pain was evaluated by daily monitoring of foot guarding behavior.

We identified three stages in the daily change of cardiovascular parameters and plasma NE level over 3 weeks following CCI. The first stage (up to 3 days after the surgery) was characterized by increased MAP and HR, especially in the daytime, even though plasma NE was unchanged and motor activity decreased. The second stage (mid first to mid second postoperative weeks) was characterized by increased daytime MAP and HR, and the animals developed punctate hyperalgesia in the affected hindpaw. An NE surge that may have been related to spontaneous pain was present 3-5 days after CCI. The third stage, which appeared after the second postoperative week, was characterized by normalized MAP and decreased HR, and increased high-frequency (0.8 - 3.0 Hz) power in pulse interval variability, which is an index of cardiac parasympathetic tone. These results

demonstrated that cardiovascular function was kept high through sympathetic and non-sympathetic activity for 2 weeks after CCI, followed by a predominance of parasympathetic tone.

1. Introduction

Complex regional pain syndrome (CRPS) is a painful neuropathic disorder that may develop after limb trauma or major peripheral nerve injury. In addition to spontaneous pain and hyperalgesia, signs of altered sympathetic outflow such as alterations in cutaneous temperature and cutaneous blood flow appear in affected areas (Baron, 2006). Some CRPS patients also obtain substantial or complete pain relief with a local sympathetic block, suggesting sympathetic activity may have a role in pain states. However, there is general agreement that activity in sympathetic neurons to the affected extremity is not increased in CRPS (Baron and Maier, 1996; Birklein et al., 1998). For instance, sympathetic nerve activity to the affected skin was found to be qualitatively normal in people with this disorder (Wallin et al., 1976; Torebjörk and Hallin, 1979; Casale and Elam, 1992). More recently, Wasner et al. (1999) reported that stimulation of cutaneous vasoconstrictor neurons did not induce a decrease of skin blood flow or temperature on the affected side but the responses to these stimuli were normal on the control side in CRPS type I. Meanwhile, it has been shown that in CRPS patients the plasma norepinephrine (NE) levels in affected limbs is significantly lower than in unaffected limbs (Drummond et al., 1991; Harden et al., 1994; Wasner et al., 1999, 2001).

In all these studies of CPRS, activity in sympathetic neurons to the affected extremity was decreased or remained the same, but never increased. Autonomic disturbances are

therefore more likely the result of reduced sympathetic activity and supersensitivity of adrenoceptors to NE (Cannon and Rosenblueth, 1949) located at nociceptors (Bossut et al., 1996), and vessels (Kurvers et al., 1998; Wasner 1999, 2001) than the result of local sympathetic overactivity. However, it remains unclear whether generalized (including cardiac) autonomic nervous function is changed in CRPS patients.

Chronic constriction injury (CCI) on the sciatic nerve of rats induces painful neuropathy that produces many of the symptoms seen in CRPS (Bennett and Xie, 1988). Guarding behavior of the nerve-injured paw, which is regarded as a sign of spontaneous pain, appears by 7 days after CCI (Bennett and Xie, 1988; Kim et al., 1997). Hyperalgesia upon mechanical or thermal stimulation of the nerve-injured paw also appears within 2-5 days and reaches peak severity in 2 weeks (Bennett and Xie, 1988; Kim et al., 1997). In addition, pronounced abnormalities of skin temperature are sometimes seen on the nerve-injured paw (Bennett and Ochoa, 1991; Wakisaka et al., 1991), although the temperature change is somewhat smaller than that in CRPS patients (Wasner et al., 2001).

In this experiment we investigated the time course of changes in cardiovascular parameters and plasma NE level in CCI animals in a neuropathic condition. Biotelemetry techniques and jugular vein catheterization for repeated blood sampling (Thrivkraman et al., 2002) allowed us to monitor these parameters continually in conscious unrestrained rats, before

and after CCI, thus eliminating concerns about handling-induced stress.

A preliminary account of this work was published in elsewhere (Sato et al., 2005).

2. Methods

All the experiments in the present study were conducted according to the Regulations for Animal Experiments in Nagoya University, and the Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions in Japan. Male Sprague-Dawley rats (250-300 g, Japan SLC) were used. The animals were housed two to three per cage under controlled temperature (22 ± 1°C) and on a 12 h light/dark cycle, and had free access to food and water. All surgical procedures described below were performed under surgically clean conditions and sodium pentobarbital anesthesia (60 mg/kg, i.p.).

2.1. CCI surgery

Experimental neuropathy in rats was produced by CCI surgery, according to a method previously described (Bennett and Xie, 1988). Briefly, the right sciatic nerve was exposed at the mid-thigh level and the nerve was then constricted with four loose ligatures using

chromic gut (4/0) spaced at about 1 mm. In the sham-operated control rats, the right sciatic nerve was exposed but not ligated.

2.2. Measurements of cardiovascular parameters

2.2.1 Telemetric monitoring of arterial blood pressure and heart rate

A telemetry system was used for monitoring parameters of cardiovascular function, namely, arterial blood pressure (BP) and heart rate (HR), of unrestrained rats. An incision was made in the abdominal median line, and a catheter, mounted with a radio-transmitter equipped with a BP transducer (Physiotel TA11PA-C4, DSI, USA) to sense instantaneous BP, was inserted into the abdominal aorta just above the bifurcation of the iliac artery. The radio transmitter-BP transducer itself was sutured to the inner surface of the peritoneal wall. On the day of the experiment, individual rat cages were placed on the top of a receiver that detected the BP radio signal (sampling rate, 100 Hz), which was relayed to a personal computer (PC) through an analog/digital converter (PowerLab/16s, ADI, UK). Mean arterial pressure (MAP) was computed by digital integration of the BP signals, and instantaneous HR was calculated from intervals between a series of BP waves using the PowerLab software. In Experiment 1, MAP and HR values were recorded every 5 minutes for 3 sec throughout the entire experimental period. In Experiment 2, MAP and HR values were

continuously recorded for 20 min during the resting condition in the daytime.

2.2.2. Analysis of pulse interval variability

Power spectrum analysis of HR fluctuations provides a quantitative means of assessing cardiovascular control system function in rats (Kuwahara et al., 1994). Moreover, the pulse interval obtained from BP waves and RR intervals obtained from electrocardiograms provides similar spectral powers in conscious rats (Daffonchio et al., 1995). In the present experiment, therefore, a continuous series of pulse intervals was computed by measuring the interval between two consecutive BP waves recorded by the telemetry system. Each 20-min recording period was divided into consecutive 60-sec periods, and the spectrum was calculated by the Fast-Fourier transform algorithm. The spectra obtained from the different 20-data sets were then averaged. Low-frequency (LF; 0.02 to 0.6 Hz) fluctuations in BP waves are considered to reflect sympathetic activity or the combined activity of the sympathetic and parasympathetic nervous systems in rats (Kuwahara et al., 1994). Recently, however, there are contradictory results showing that LF spectral components are not always suitable markers for the prevailing sympathetic nerve activity (Daffonchio et al., 1995; Stauss et al., 1995). In the present experiment, therefore, we analyzed only high-frequency (HF) spectral components (0.8 to 3.0 Hz) of pulse intervals, which are generally agreed to reflect parasympathetic tone (Cerutti et al., 1991; Kuwahara et al.,

1994), and which we considered to be an indicator of cardiac parasympathetic tone.

2.3. Measurement of plasma norepinephrine level

A surgical technique was introduced to catheterize the external jugular vein for repeated blood sampling from the vena cava close to the right atrium in unrestrained rats (Thrivkraman et al., 2002). Briefly, a polyethylene catheter filled with heparinized saline was inserted into the right external jugular vein so that the tip lay close to the right atrium. The catheter was then tunneled subcutaneously and exteriorized at the dorsal neck. Rats were given 24 hours to recover from the surgery. On the day of the sampling, blood (0.2-0.4 ml per sample) was collected into iced heparin-treated microcentrifuge tubes, centrifuged and kept frozen until assay. An amount of plasma NE was assayed by batch alumina extraction, followed by high performance liquid chromatography with electrochemical detection (Type 300, EICOM, Japan). The areas under the NE signal (retention time: 8-9 min) in the chromatogram curves were calculated with the PC-software (PowerChrom v.2.1J, ADI, Japan), and converted into amount of NE.

2.4. Measurement of nociceptive behaviors

2.4.1. Punctate hyperalgesia

Punctate hyperalgesia was measured by the von Frey hair test. Each rat was placed individually beneath an inverted transparent plastic cage (11 x 17 x 11 cm) with a wire mesh bottom. Hand-made von Frey hairs (diameter, 0.5 mm) were applied perpendicularly to the plantar surface of the nerve-injured paw through the wire mesh with sufficient force to cause slight bending against the paw, and held for 2 sec or until the rat withdrew its paw. The hairs were applied in the order of increasing bending force (from 34.3 to 739.7 mN), with each applied 5 times at intervals of 2-3 sec to different parts of the mid-plantar glabrous skin. The strength of the first hair in the series that evoked at least 1 positive withdrawal response among the 5 trials was designated the pain threshold.

2.4.2. Spontaneous pain

To assess spontaneous pain (ongoing pain without apparent external stimuli) in the neuropathic rats, we observed the animals' guarding behavior (Bennett and Xie, 1988) in a natural setting without intervention from the experimenter. Each CCI and sham-operated rat was placed individually beneath an inverted transparent plastic cage (11 x 17 x 11 cm) with a wire mesh bottom. After 5 min of adaptation, the cumulative time during the next 5 min that the rat held its foot off the floor was recorded. However, foot lifts associated with locomotion or body repositioning were not counted.

2.5. Measurement of motor activity

Motor activity was quantified using an ANIMEX activity meter (AUTO MK-110, Muromachi Kikai, Japan) set to maximum sensitivity. Every movement of the rats produced a signal from the change in inductance and capacity of the apparatus resonance circuit. Then signals were automatically relayed to the A/D converter connected to a PC, and integrated every 5 min.

2.6. Experimental protocol

The present study consisted of three experiments.

2.6.1. Experiment 1: Short-term effect of CCI on the circadian changes of cardiovascular parameters and motor activity

First, the short-term effect of CCI on the circadian changes of cardiovascular parameters and motor activity was tested. For this purpose, seven CCI and seven sham-operated rats were kept individually in cages (45 x 25 x 20 cm) in a temperature-controlled room (22 ± 1°C) with lights on from 7:00 to 19:00 h for 2 days before surgery and 4 days

postoperatively. Instantaneous MAP and HR values were sampled (100 Hz) every 5 minutes for 3 sec throughout the entire experimental period. In six CCI and seven sham-operated rats, motor activity was also recorded continuously (in 5 min bins). Daily changes in daytime and nighttime MAP, HR, and motor activity were examined by averaging the values during the daytime and nighttime separately.

2.6.2. Experiment 2: Long-term effect of CCI on the cardiovascular parameters in resting condition, punctate hyperalgesia, and motor activity

Secondly, the long-term effect of CCI on the cardiovascular parameters in resting rats was evaluated using a group of rats different from those in Experiment 1 (n = 18). For this purpose, the MAP, HR, and HF power during the resting condition were measured 2 and 1 days before, and 4, 7, 11, 15, and 19 days after the CCI (n = 8) or sham surgery (n = 5). Control rats without the surgeries were also used (intact rat, n = 5). Recordings were carried out in a sound attenuated, temperature- (22 ± 1.0°C) and humidity-controlled (55 ± 5%) climate room. To minimize effects attributable to circadian alterations, these cardiovascular parameters were measured between 10:00-14:00 h. The animals were monitored visually by the investigator throughout the recording session, and BP data were continuously recorded for 20 min while the rats remained in a lying or sitting position without any body movements, such as grooming, eating, drinking, stretching, exploring, and rearing.

From this BP wave, MAP, HR and HF power were computed as described above. In these CCI rats, measurement of withdrawal threshold to stimulation with von Frey hairs in the affected paw was also carried out on the same day as the cardiovascular recordings, and the data were compared with those of another group of six sham-operated rats. In addition, continuous recording of motor activity was carried out up to 19 days after the CCI surgery in the CCI group in Experiment 1 (n = 6).

2.6.3. Experiment 3: Effects of CCI on the plasma NE concentration and spontaneous pain behavior in resting rats

Thirdly, the effect of CCI on the plasma NE concentration in resting rats was tested (n = 16). All daily blood samplings were carried out between 10:00-12:00 h to minimize effects of circadian patterns. During blood sampling, rats were kept unrestrained and rested quietly in their own cage (45 x 25 x 20 cm) in a temperature-controlled room (22 ± 1°C). The animals were monitored visually by the investigator, and the resting condition was identified visually as times when the animal maintained a lying or sitting position without any body movements. Although the catheter was flushed daily with heparinized saline to maintain its patency, daily blood samplings could not be continued for longer than several days. CCI rats were therefore divided into three groups according to blood-sampling period. These were group 1 (n = 4): 1 day before, on the day of (day 0, just before CCI surgery) and 1, 2,

and 3 days after the CCI surgery; group 2 (n = 6): 1 day before, on the day of (day 0, just before CCI surgery), and 3, 4, 5, and 6 days after the CCI surgery; and group 3 (n = 6): from 6 to 13 days after the CCI surgery. In the sham-operated rats (n = 5), blood samplings were carried out 1 day before, on the day of (just before surgery), and 3, 4, 5, and 6 days after the sham surgery. In the rats of group 2 and the sham-operated rats, measurement of guarding behavior was also carried out following a rest period of at least 60 min after the blood sampling, and the time course of spontaneous pain was evaluated.

2.7. Data analysis

Group data are presented as mean ± SEM. One-way or two-way repeated-measures analysis of variance (ANOVA) and Tukey's post hoc tests were utilized to analyze the influence of surgery (sham or CCI) and days after surgery on MAP, HR, and motor activity. The two-sample t test was employed to compare the values of control and surgical groups. The Friedman followed by Dunn tests were used to compare the non-parametric data from the von Frey hair test. Differences were considered statistically significant at the $P < 0.05$ level.

3. Results

3.1. Experiment 1: Short-term effect of CCI on the circadian changes of cardiovascular parameters and motor activity

Before CCI, both MAP and HR showed high levels during the night because the rat is a nocturnal animal and motor activity occurred primarily during the dark. Changes in MAP, HR and motor activities during daytime and nighttime showed different patterns after the CCI (Fig. 1A, B and C). In the CCI rats, MAP in the nighttime on postoperative days 1 (PO1) – 4 (PO4) was significantly higher than the average value 1 and 2 days before surgery (PRE)(Fig. 1A). Increments of MAP in the daytime on these postoperative days were also apparent. The HR in the daytime also increased right after the CCI (Fig. 1B). Specifically, the mean HR in the daytime on PO1 – PO3 was significantly higher than the PRE value. In contrast, the mean nighttime HR was not influenced by the CCI (Fig. 1B).

Motor activity also had a clear diurnal rhythm with higher activity in the nighttime during the pre-CCI period (Fig. 1C). It should be noted that motor activity in the nighttime became sporadic and clearly dropped during the first day after the CCI, when nighttime MAP was higher than the preoperative level (Fig. 1A), and then gradually returned to baseline by PO4. Motor activity in the daytime, on the other hand, was not clearly influenced by the CCI. These observations indicate that the increments of MAP and HR in the first 3 days after the CCI are not due to increased motor activity.

Sham surgery also induced short-term increases in MAP (Fig. 1A) and HR (Fig. 1B) in the daytime and/or nighttime, and a decrease in the nighttime motor activity (Fig. 1C). Changes in these parameters after sham surgery were similar to those after CCI. Two-way repeated-measures ANOVA and post hoc analyses also revealed that the mean daytime HR of sham rats (Fig. 1B) began to return toward the initial values somewhat sooner than CCI. The HR value on PO3 was significantly lower than that of CCI rats. In addition, as will be seen in the last paragraph on spontaneous pain behavior, the animals did not exhibit guarding behavior of the affected hindpaw for the first 3 days after the CCI surgery. Thus, the increments of MAP and BP in the first 2-3 days after the CCI can be explained quite naturally as the result of short-term autonomic reaction to postoperative stress rather than CCI in particular.

In the next experiment, we focused on the long-term effect of CCI on cardiovascular autonomic parameters, motor activity, and pain behavior (punctate hyperalgesia). For this purpose, measurements were done during a later period after the CCI (PO4 – PO19), when the short-term effect of surgical stress could be excluded.

3.2. Experiment 2: Long-term effect of CCI on cardiovascular parameters in resting condition, motor activity, and punctate hyperalgesia in rats

Figure 2 shows a typical example of original 20-min records of BP with the mean value (white trace), and HR in the daytime from a single resting rat after the CCI. BP increased on PO4 – PO11, and returned to the preoperative level on PO19. HR increased on PO4 – PO7, and decreased on PO15 and PO19. Average time courses of MAP and HR in the daytime after the CCI or sham surgery compared with those of intact control rats are summarized in Fig. 3. Two-way repeated-measures ANOVA revealed that in the sham-operated as well as the control rats, these cardiovascular parameters during 20-min resting condition did not significantly change throughout the entire observation period. In comparison, resting CCI rats displayed a significant increase in the MAP on PO4, and this increasing effect continued up to PO11, but disappeared after PO15 (Fig. 3A). The MAP value of the CCI rats on PO4, PO7, and PO11 was significantly higher than that of intact control rats. The HR of resting rats in the daytime also rose in the early postoperative days after the CCI (Figs. 2 and 3B): The value of CCI rats on PO4 was significantly higher than that of control rats, and that of the preoperative value. Following this increment, HR gradually declined, and on both PO15 and PO19 it was even lower than the preoperative value (Figs. 2 and 3B). The HR value of CCI rats on these postoperative days was significantly lower than that of the control rats. In contrast, sham-operated rats showed no change in MAP and HR during the entire observation period (Figs. 3A and B).

Figure 4 shows the time course of spectral profiles of pulse interval variability throughout 20-min resting period in the same CCI rat as shown in Fig. 2, while the average spectral data are reported in Fig. 5. Two-way repeated-measures ANOVA and Tukey's post hoc tests indicated that the HF power started to increase on 11 days post-CCI and this increment lasted up to PO19. The average HF power of the CCI rats on these later postoperative days was significantly larger than that of the control rats, suggesting cardiac parasympathetic activation in the later postoperative days.

Many research groups have found that CCI surgery on the sciatic nerve evokes spontaneous pain behaviors, and relatively long-duration allodynia and hyperalgesia in the affected paw (Bennett and Xie, 1988; Attal et al., 1990; Kim et al., 1997; Dowdall et al., 2005). The time course of postoperative painful behaviors of CCI rats used in the present experiment was identical to those reported previously. Namely, by PO7 all the CCI rats showed shaking and licking, and often held the hindpaw of the affected side off the floor much of the time, which are behaviors suggestive of spontaneous pain induced by the CCI. The withdrawal threshold was decreased on the 4th day post-CCI and remained significantly lower than in the pre-CCI period up to PO19 (Fig. 6A). Sham surgery, on the other hand, did not change the withdrawal threshold in any of 6 rats (Fig. 6B).

In contrast, although the average value of motor activity during the nighttime was clearly

suppressed during the first 3 days after CCI as reported above (3.1, second paragraph), it (during both night- and day-time) had returned to the normal level on PO4 – PO19 (Fig. 7). These observations indicate that the changes in MAP, HR, and HF power during PO4 – PO19 are not due to changed motor activity.

3.3. Experiment 3: Effects of CCI on plasma NE concentration and spontaneous pain behavior

The above-reported BP and HR increases suggest the existence of sympathetic activation during early postoperative period following CCI, but this may be limited to the cardiovascular sympathetic system. Therefore, to see whether sympathetic nerve activity in general is increased, in the third series of experiments we measured circulating NE concentration each day after the CCI as an indicator of generalized sympathetic activity in rats (Goldstein et al., 1983). Time courses of plasma NE level in individual rats under the resting condition in the daytime are shown in Fig. 8. There were no apparent changes in the NE level 1-2 days after the CCI (group 1), when stress reaction to the surgical procedures was expected to occur. Afterward, plasma NE concentration in all rats markedly increased, reaching a peak on PO3 or PO4 (group 2) and then returning to the preoperative level by PO7, after which it remained steady (group 3).

The time courses of plasma NE concentration from one day before to 6 days after the CCI and sham surgeries are compared in Fig. 9. The averaged values for CCI rats were obtained from the data for group 2 of the NE series (Fig. 8). In the CCI rats there were relatively wide standard errors in the average NE concentration, because each rat had a peak value on different postoperative days (Fig. 8). Even with these variations, however, the average NE concentration on PO4 and PO5 was significantly larger than the pre-CCI level. This augmenting effect of CCI seemed to last until PO6, although the change was not significant. Sham surgery, on the other hand, did not change plasma NE concentration in any of five rats (Fig. 9). Thus, the average NE concentration of CCI rats was significantly higher than that of sham-operated rats on PO4 through PO6.

Figure 10 shows the time course of guarding behavior of CCI rats of group 2 of the NE series (n = 6). The duration of foot lifts in individual CCI rats was increased for a short period on PO4 – PO6, except for rat (a), which showed no guarding behavior during the observation period. On average, the duration on PO5 and PO6 was significantly longer than the preoperative value (0 sec). This onset of spontaneous pain behavior roughly corresponds to the time of plasma NE increase (Fig. 9). Rat (a) showed a relatively small increase in plasma NE concentration [(a) in Fig. 8] in comparison with the others. The sham surgery did not induce guarding behavior (data not shown).

4. Discussion

One of the major findings of the present study is that, up to 2 - 3 days after CCI, MAP in the nighttime (active period), and MAP and HR in the daytime (resting period) increased without significant change in plasma NE concentration. Since plasma NE level reflects general sympathetic activity in rats (Goldstein et al., 1983), our present findings suggest that the increments in MAP and HR for several days after CCI were not due to generalized (including cardiac) sympathetic nerve activity. It would seem that such increments of MAP and HR may be caused by surgical stress including increased nociceptive input from the surgical site. This hypothesis is supported by the fact that sham surgery also increased MAP and HR, and decreased motor activity in the nighttime during the first few days after the surgery.

The second major finding is that there was an abrupt increase in plasma NE level 3-6 days after CCI when punctate hyperalgesia developed and spontaneous pain behavior appeared, and MAP and HR were still high. Since the plasma NE did not increase after sham surgery, it is unlikely that the postoperative stress induced an increase in the sympathetic nerve activity that resulted in the NE surge on PO3 – PO6. One alternative possibility is that the presence of pain (especially spontaneous pain) after CCI may have activated the sympathetic nervous system and consequently increased the circulating NE level. In line

with this, the time course of the increased NE level paralleled the time course of the appearance of spontaneous pain behavior in our study. Another possibility is that the release of NE from the degenerating sympathetic fibers in the injured sciatic nerve caused the increase in plasma NE. However, this is unlikely because the number of fibers in the sciatic nerve in relation to the total number of sympathetic postganglionic fiber in the body is only 10 to 20% (Baron et al., 1988). A return to normal levels of plasma NE level in the second week after CCI is compatible with the observation in the spinal nerve ligation model by Raja et al. (1995) that the plasma NE level measured in the affected limb under anesthesia decreased 2-4 weeks postoperatively.

Interestingly, MAP and HR of resting rats was already elevated one day after CCI, and high MAP lasted 10 days thereafter. Our new data therefore demonstrated for the first time that basal BP of rats remains high during the first 2 weeks after CCI. Since plasma NE concentration promptly returned to the preoperative level after PO7, it is obvious that the sustained increase of MAP in the second week after CCI is not due to sympathetic nerve hyperactivity. Previously, it was demonstrated that CCI rats show significant and striking disruption to sleep-wake cycles such as decreased slow-wave sleep and increased wakefulness or arousal during both light and dark cycles, particularly when the condition continues for at least three weeks (Andersen and Tufik, 2003; Monassi et al., 2003). Therefore, the increase in MAP over the first two weeks after the CCI may have been a

result of sleep disturbances induced by the surgery, although the duration of MAP change was shorter than that in those previous experiments.

The third major finding is that HF power, which is an indicator of cardiovascular parasympathetic tone, increased at the third week after CCI when NE level was normal, and the punctate hyperalgesia reached maximum severity. It is possible that a CCI-induced neuropathic condition may induce cardiovascular parasympathetic activation in animals. However, we have no definite information on this point and there is room for further investigation. In any case, our present results are noteworthy because no studies have ever examined, to our knowledge, the time course of cardiovascular parasympathetic tone in any animal models of chronic pain.

It is well known that partial nerve injury or inflammatory tissue damage induces a change in peripheral nociceptors, making them excitable by sympathetic activity or adrenergic substances (Hu and Zhu, 1989; Sato and Perl, 1991; Sato et al., 1993; Bossut and Perl, 1995). This excitation is mediated by increased presence of alpha-adrenoceptors (Kurvers et al., 1998; Birder and Perl, 1999) and has a time course reminiscent of experimental denervation supersensitivity (Cannon and Rosenblueth, 1949). It has been suggested that these additional adrenergic receptors make nociceptors and other primary afferent neurons excitable by local or circulating NE and epinephrine (O'Halloran and Perl, 1997).

Additionally, such adrenergic sensitive sensory fibers were suggested to have an important role in the generation of sympathetic-activity dependent pain states (Neil et al., 1991). In the present experiment, circulating NE level abruptly increased 3-6 days after CCI and then dropped to the normal level when the punctate hyperalgesia reached peak severity. Therefore, it seems reasonable to assume that adrenergic supersensitivity would develop in nociceptive afferents in CCI rats independently of the increase in NE secretion, or perhaps afterward during a period of relative local reduction in NE secretion.

In summary, the present study showed that there were three stages in daily change of cardiovascular autonomic parameters and plasma NE level after CCI during the 3-week follow-up period. The first stage, which lasts for 3 days after the surgery, is characterized by increments of MAP and HR, especially in the daytime. These changes may be cardiovascular autonomic responses to the surgical stress in general rather than to CCI in particular. The second stage, encompassing most of the first and second postoperative weeks, is characterized by increments of MAP and HR during the resting condition in the daytime. This may be partially due to sleep disturbances in the neuropathic condition. An NE surge was present during the first few days (PO3 – PO6) of the second stage, when the spontaneous pain behavior was observed. The third stage, which appears after the second postoperative week when the punctate hyperalgesia reached peak severity, is characterized by normal MAP and decreased HR. In this stage, cardiovascular

parasympathetic activity might have increased.

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Figure legends

Fig. 1

Time courses of changes in cardiovascular parameters and motor activity in the daytime and nighttime after CCI or sham surgery.

Mean arterial pressure (MAP; A), heart rate (HR; B) and motor activity (C) in CCI (circles) and sham-operated rats (triangles) in the daytime (open symbols) and nighttime (solid symbols) are shown (mean ± SEM). The horizontal axis shows days after CCI or sham surgery. PRE indicates average value 1 and 2 days before surgery. In A and B, n = 7 in each group; in C, n = 6 in CCI rats, n = 7 in sham rats. Two-way repeated-measures ANOVA revealed a significant day-effect on both daytime and nighttime MAP [daytime: $F(4, 48) = 19.21$ and $P < 0.0001$, nighttime: $F(4, 48) = 16.05$ and $P < 0.0001$], daytime HR [$F(4, 48) = 6.52$ and $P = 0.00028$], and nighttime motor activity [$F(4, 44) = 10.24$ and $P < 0.0001$]. In the daytime HR, a significant surgery-effect was also observed [$F(1,12) = 8.22$ and $P = 0.014$]. *$P < 0.05$, compared with each PRE value (Tukey's post hoc test); †$P < 0.05$, compared with that of sham-operated rat (two sample t-test).

Fig. 2

Original 20-min recordings of arterial pressure (BP, upper graphs) with the mean value (white trace), and heart rate (HR, lower graphs) from a single rat in resting condition 1 day

before, 4, 7, 11, 15, and 19 days after the CCI (postoperative days shown at the top of each recording).

Fig. 3

Time courses of cardiovascular parameter change in resting rats after CCI.

Mean arterial pressure (MAP; A) and heart rate (HR; B) in CCI (solid circles; n = 8), sham-operated (triangles; n = 5), and intact control (open circles; n = 5) rats under 20-min resting condition in the daytime (mean ± SEM). The horizontal axis shows days after CCI or sham surgery. PRE indicates average value 1 and 2 days before surgery. Two-way repeated-measures ANOVA revealed a significant day-effect on both MAP [$F(5, 75) = 4.50$ and $P = 0.0012$] and HR [$F(5, 75) = 10.09$ and $P < 0.0001$], but no surgery-effect [MAP: $F(2,15) = 2.37$ and $P = 0.13$, HR: $F(2, 15) = 0.86$ and $P = 0.44$]. CCI but not sham surgery significantly changed these cardiovascular parameters. *$P < 0.05$, compared with each PRE value (Tukey's post hoc test); †$P < 0.05$, compared with that of each intact rat (two sample t-test).

Fig. 4

Sequential spectral profiles of pulse intervals after CCI.

Analysis was carried out on blood pressure recording taken from the same CCI rat as shown in Fig. 2. The horizontal axis shows frequency of pulse interval variability. Vertical

axis shows power spectrum. LF: low-frequency band (0.02 – 0.6 Hz), HF: high-frequency band (0.8 – 3.0 Hz) in which the main parasympathetic activity-related changes were observed. Numbers shown at the top of each graph indicate postoperative days (before: 1 day before surgery).

Fig. 5

Time-dependent change of high frequency (HF) power in pulse interval variability in resting rats after CCI.

Graph shows changes in CCI (solid circles; n = 8), sham-operated (triangles; n = 5) and intact control (open circles; n = 5) rats under 20-min resting condition in the daytime (mean ± SEM). The horizontal axis shows days after CCI or sham surgery. PRE indicates average value 1 and 2 days before surgery. Two-way repeated-measures ANOVA revealed significant both surgery- and day-effects on HF power [$F(2,15) = 3.92$ and $P = 0.04$ for surgery, $F(5, 75) = 13.44$ and $P < 0.0001$ for day]. CCI but not sham surgery significantly changed the HF power. *$P < 0.05$, compared with each PRE value (Tukey's post hoc test). †$P < 0.05$, compared with that of each intact rat (two sample t-test).

Fig. 6

Change in withdrawal threshold after CCI and sham surgeries.

The data were obtained from CCI (A, n = 8) and sham rats (B, n = 6). Data are presented as

box [median ± interquartile range (IQR)] and whiskers (5 and 95 percentile values). Average withdrawal threshold was significantly changed by CCI surgery (Friedman test, P = 0.0012). CCI surgery significantly lowered the threshold value below the preoperative value during post CCI days 4 - 19 (PRE in A). In contrast, sham surgery did not alter withdrawal threshold on any postoperative days (B). *P < 0.05, compared with PRE (Dunn test).

Fig. 7

Effect of CCI on the circadian variation of motor activity.

Averaged counts of motor activity during daytime (open circles) and nighttime (solid circles) before and after CCI surgery (n = 6). Error bars are omitted for clarity. One-way repeated-measures ANOVA revealed significant day-effect during nighttime [F (19,209) = 5.63 and P = 0.0001]. *P < 0.05, compared with average nighttime value of 1 and 2 days before CCI (Tukey’s post hoc test).

Fig. 8

Time courses of plasma norepinephrine (NE) change in individual CCI rat.

The horizontal axis shows days after CCI and the vertical axis plasma NE concentration. Animals were divided into three groups according to a blood-sampling period (see methods). The data (a) was obtained from the same rat as the rat marked with (a) in Fig. 10. As a whole, plasma NE levels were sharply increased 3-5 days postoperatively.

Fig. 9

Effect of CCI (n = 6) or sham surgery (n = 5) on plasma norepinephrine (NE) concentration in resting rats.

The horizontal axis shows days after CCI or sham surgery and the vertical axis plasma NE concentration. Two-way repeated-measures ANOVA revealed significant surgery- and day-effects on the NE concentration [$F(1, 9) = 38.4$ and $P = 0.00016$ for surgery, $F(5, 45) = 5.05$ and $P = 0.0009$ for day]. CCI but not sham surgery significantly increased NE level. *$P < 0.05$, compared with 0 day (Tukey's post hoc test). †$P < 0.05$, compared with that of each sham-operated rat (two sample t-test).

Fig. 10

Time of CCI rats spent in guarding behavior.

Individual values (dotted lines) and the averages (mean ± SEM, solid line, n = 6) of foot lift duration are shown. The data were obtained from group 2 of NE series in Fig. 8. The data (a) were obtained from the same rat as the rat marked with (a) in Fig. 8. One-way repeated-measures ANOVA revealed a significant day-effect on the foot lift duration [$F(5,30) = 2.76$ and $P = 0.036$]. Average duration on postoperative days 5 and 6 was significantly longer than the preoperative value (0 sec). *$P < 0.05$, compared with 0 day (Tukey's post hoc test).

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