Published in final edited form as:

Pain. 2009 December ; 146(3): 293–300. doi:10.1016/j.pain.2009.07.040.

Baroreceptor Reflex is Suppressed in Rats that Develop Hyperalgesia Behavior after Nerve Injury

Geza Gemes, M.D.^{a,e}, Marcel Rigaud, M.D.^{a,e}, Caron Dean, Ph.D.^{b,f}, Francis A. Hopp, MSEE^{d,f}, Quinn H. Hogan, M.D.^{*,c,f}, and Jeanne Seagard, PhD.^{c,f}

^aPost-doctoral Fellow, Medical College of Wisconsin, Milwaukee, Wisconsin

^bAssociate Professor, Medical College of Wisconsin, Milwaukee, Wisconsin

^cProfessor, Department of Anesthesiology, Medical College of Wisconsin, Milwaukee, Wisconsin

^dEngineering Technician, Medical College of Wisconsin, Milwaukee, Wisconsin

^eResident, Department of Anesthesiology and Intensive Care Medicine, Medical University of Graz, Graz, Austria

^fDepartment of Anesthesiology, Zablocki VA Medical Center, Milwaukee, Wisconsin

Abstract

The baroreceptor reflex buffers autonomic changes by decreasing sympathetic activity and increasing vagal activity in response to blood pressure elevations, and by the reverse actions when the blood pressure falls. Because of the many bidirectional interactions of pain and autonomic function, we investigated the effect of painful nerve injury by spinal nerve ligation (SNL) on heart rate (HR), blood pressure (BP) and their regulation by the baroreceptor reflex. Rats receiving SNL were separated into either a hyperalgesic group that developed sustained lifting, shaking and grooming of the foot after plantar punctate nociceptive stimulation by pin touch, or into a group of animals that failed to show this hyperalgesic behavior after SNL. SNL produced no effect on resting BP recorded telemetrically in unrestrained rats compared to control rats receiving either skin incision or sham SNL. However, two tests of baroreceptor gain showed depression only in animals that developed sustained hyperalgesia after SNL. Animals that failed to develop hyperalgesia after SNL were found to have elevations in HR both before and for the first 4 days after SNL, and HR variability analysis gave indications of decreased vagal control of resting HR and elevated sympatho-vagal balance at these same time intervals. In human patients, other research has shown that blunted baroreceptor reflex sensitivity predicts poor outcome during conditions such as hypertension, congestive heart failure, myocardial infarction, and stroke. If baroreceptor reflex suppression is also found in human subjects during chronic neuropathic pain, this may adversely affect survival.

1. Introduction

It is well recognized that acute increases in afferent neuronal traffic along nociceptive pathways produces heightened circulatory performance [29]. Even during general anesthesia, high

*Corresponding author. Address: Department of Anesthesiology, Medical College of Wisconsin, Room M4280, 8701 Watertown Plank Rd. Milwaukee, WI, USA, 53226; Tel.: 414 456-5727; fax 414 456-6507. qhogan@mcw.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

The authors have no financial or other relationships that constitute a conflict of interest.

intensity mechanical and thermal stimuli elevate both the arterial blood pressure (BP) and heart rate (HR) [1], and may precipitate adverse outcomes such as cardiac dysrhythmia, myocardial ischemia or congestive heart failure. Additionally, higher-level integrative processes that regulate BP and HR are influenced by painful stimulation. For instance, acute activation of nociceptors reduces baroreflex sensitivity and attenuates baroreflex-induced bradycardia [7, 27]. Little is known, however, about the consequences of chronic pain upon circulatory homeostasis. Since plasticity is a common feature in nociceptive systems, it would be unsafe to extrapolate from these observations on hemodynamic responses during acute painful stimulation to those that may occur with pain that persists for days or weeks, and either accommodation or potentiation of acute responses might be expected in the context of chronic pain.

Only a few initial studies of the long-term effects of sustained pain on cardiovascular control have been performed. In rats, chronic constriction injury of the sciatic nerve produces an elevation of arterial BP and HR in the initial postoperative period, but as pain behavior evolves in the following weeks, the BP returns to normal and sustained bradycardia develops, accompanied by HR variability (HRV) changes indicative of parasympathetic activation [15]. However, baroreflex sensitivity was not examined in that study. In humans, baroreflex function is normal during reflex sympathetic dystrophy in adolescents [23]. However, adults with fibromyalgia [12] show diminished orthostatic tolerance, accompanied by reduced sympathetic activation and vagal withdrawal, indicative of compromised cardiovascular reflex control. There has been no examination of cardiovascular autonomic control in human subjects with chronic pain due to nerve damage.

The lack of knowledge about cardiovascular regulation during neuropathic pain in either an animal or human model led us to investigate if there were changes in BP, HR, or baroreflex sensitivity induced by chronic pain from a peripheral nerve injury. These parameters were observed in rats for the three week period following spinal nerve ligation (SNL) during which neuropathic pain develops [14]. Sham surgery control groups were included to identify nonspecific contributions of handling, exposure to anesthesia, and non-neural tissue injury. Since HR, BP, and baroreceptor reflex activity are strongly depressed during application of anesthesia [32], and may also be influenced by the stress of restraint, a telemetric technique was employed that allows the animal subjects to be awake and freely active during recording.

2. Experimental Procedures

2.1 Experimental animals

A total of 35 male Sprague-Dawley rats (150–175 g at the initiation of the protocol) were obtained from a single vendor (Taconic Farms, Inc., Hudson, New York) for use in these studies. Animals were housed individually in a room maintained at $22 \pm 0.5^\circ\text{C}$ and constant humidity ($60 \pm 15\%$) with an alternating 12-h light-dark cycle. Food and water were available *ad lib.* throughout the experiments. All procedures were approved by the Animal Care and Use Committees of the Zablocki VA Medical Center and Medical College of Wisconsin (Milwaukee, Wisconsin).

2.2 Surgery

BP was monitored by telemetry before and after SNL. A PA-C10 transmitter (Data Sciences International (DSI), St. Paul, MN) was implanted during anesthesia with isoflurane (1.5–2.0%) in oxygen. After making a small left inguinal incision, the cannula (0.43 mm outer diameter polyethylene) attached to the transmitter was inserted into the left femoral artery, with care taken to avoid manipulation of the adjacent femoral nerve, and the transmitter fixed in a subcutaneous pocket on the left flank of the rat. A redundant loop in the cannula allowed for

growth of the rat. The incision was closed with 3-0 silk suture. Following surgery, animals were treated with buprenorphine (0.05 mg/kg subcutaneous) for surgical pain.

Four to five days after the implantation surgery, the BP for each animal was recorded as pre-SNL control (detail below). Animals within a cohort that arrived at the laboratory together were randomly allocated to surgery groups such that investigators were unaware of group membership. For SNL, rats were anesthetized with isoflurane (1.5–2.0%) in oxygen, the back was shaved, and the right lumbar paravertebral region was exposed through a midline posterior incision. After subperiosteal removal of the sixth lumbar transverse process, both the right fifth and the sixth lumbar spinal nerves were tightly ligated with 6-0 silk suture and transected distal to the ligation. To minimize non-neural injury, no muscle was removed, muscles and intertransverse fascia were incised only at the site of the two ligations, and articular processes were not removed. The lumbar fascia was closed by 4-0 resorbable polyglactin suture, and the skin was closed with three staples. Two control conditions were employed. Sham SNL surgery (ShSNL) consisted of an identical procedure except that the nerves were not ligated or sectioned after exposure. A skin incision (SI) group had only anesthesia, a lumbar midline skin incision, and skin closure. No postoperative analgesic was provided for these procedures in order to avoid possible interference with the development of the chronic pain phenotype after SNL.

2.3 Measurement of HR and MAP at rest

In order to identify the influence of peripheral nerve injury, telemetric recording of BP was performed at rest on the day prior to surgery (day -1) and days 1 through 9 and 12, 16, and 20 following surgery. Blood pressure was recorded at a sampling rate of 500Hz using the DSI PhysioTel telemetry system connected to a PC computer built in-house, and data were stored for later analysis. Heart rate was determined from the unfiltered BP trace using the DSI Dataquest A.R.T.TM software system. To obtain baseline HR and BP, BP was recorded over a ten minute resting period with no animal ambulation, verified by the PA-C10 transmitter which also detects transmitter movement. Recording sessions were scheduled between 8AM and 10AM, which minimized diurnal variation and daytime observations are optimal for identifying effects of injury on cardiovascular parameters [15]. Blood pressure and HR were averaged over the 10min recording session to obtain mean values for these parameters for each recording day.

2.4 Measurements of baroreceptor sensitivity by the spontaneous baroreceptor method

Resting recordings were continued for at least 60 min so that 40 min of quiet resting pressure could be obtained for analysis of spontaneous baroreflex gain using the DSI Dataquest A.R.T.TM software system. We ensured that BP recordings were obtained during times of minimal movement in order to avoid the elevated pressure that accompanies activity. Full, non-averaged data stored as text files were used later for determination of baroreceptor sensitivity using HemoLab software (<http://www.intergate.com/~harald/HemoLab/HemoLab.html>) of Stauss [35,36]. The sequence method, described by Bertinieri *et al.* [5,6], identifies sequences of three or more heart beats where systolic BP and pulse interval change in the same direction, that is both systolic BP and inter-beat interval (ibi) increase (up sequences) or decrease (down sequences). To account for the time delay inherent in the reflex [26], the systolic pressure of a beat was linked with the ibi four beats later (Fig. 1A). After converting the ibi into an instantaneous HR by the formula $HR = 60/ibi$ (HR is in beats per minute, ibi is in seconds), HR was plotted against systolic BP and a linear regression was calculated for each sequence (Fig. 1B). The slopes for all sequences, combining up and down sequences (approximately 17 in each recording session) that had a R^2 (squared correlation coefficient) of 0.8 or better, were averaged for each time period to determine the gain of the baroreceptor reflex.

2.5 Measurements of baroreceptor function by the phenylephrine pressor test

At the end of the 21d testing period, a separate measurement of baroreflex sensitivity was done by the standard Oxford pharmacological method [34] in which a bolus of phenylephrine is used to produce an acute elevation of BP and induce a reflex decrease in HR. Rats were anesthetized with isoflurane (1.5–2.0%) in oxygen and a catheter was placed into the left subclavian vein for infusion of phenylephrine. After catheter placement, the anesthetic was turned to the lowest level which also prevented a response to toe pinch to minimize the effects of anesthesia as much as possible (0.75 to 1.0% isoflurane). While BP was recorded using the DSI system, a low dose (0.8–1.2 μ g) of phenylephrine was injected IV to produce a BP increase of 50 – 60 mmHg. Baroreflex sensitivity was determined as the ratio of the maximal change in HR (baseline HR – minimum HR) divided by the maximal change in systolic BP (maximum SBP – baseline SBP).

2.6 Heart rate variability determination

Power spectral analysis of heart rate was used to determine HRV on days -1, 1, 4, 8, 12, 16, and 20 from blood pressure data using DSI Dataquest A.R.T. software. A cubic algorithm was used to interpolate BP data sampled at 500 Hz to 2 KHz. BP was used to calculate an ibi series from three 5-minute segments, each with 5 overlapping (50%) sub-segments of 512 points, which were taken from the same quiet resting periods that were used to determine SBS. The ibi values were interpolated at 50 Hz (cubic algorithm), detrended, and the mean was suppressed to create data points equally spaced in time for further analysis. Utilizing a Hanning window, power was calculated for each data set over the frequency ranges of 0.25–1 Hz (low frequency, LF) and 1–3 Hz (high frequency, HF), with results from the three segments averaged for the final determination of density within each frequency band. LF and HF powers were normalized and expressed as percentages of total power (LF + HF power) and the LF/HF ratio was calculated from these values.

Power spectral analysis determination of HRV yielded values for LF and HF power as percents of total power and the LF/HF ratio. It is generally accepted that HF power is a reflection of vagal modulation of heart rate [10,21], however there is disagreement as to whether LF power is a good reflection of sympathetic modulation of heart rate. Studies have found that a significant amount of power in the LF band is due to vagal contributions, and therefore the LF/HF ratio is thought to better represent the sympathovagal balance [10]. Thus, an increase in the LF/HF ratio indicates a change to a relatively higher sympathetic *versus* vagal cardiac modulation, while a decrease in the LF/HF ratio reflects the opposite.

2.7 Behavioral evaluation of hyperalgesia

Testing was performed on the day preceding surgery (day -1) and on 1, 3, 7, 14, and 21 days after surgery, with the animals placed individually in clear plastic enclosures (10 × 25 cm) upon a 1/4-in wire grid. After the animal ceased exploratory activity, the point of a 22g spinal anesthesia needle was applied to the center of the right paw with enough force to indent the skin but not puncture it. Responses were of two types, either a very brisk simple withdrawal with immediate return of the foot to the cage floor, or a sustained elevation with shaking, licking and grooming [14], which we hereafter refer to as a hyperalgesia-type response. The response type was noted for each of 5 applications separated by at least 10 seconds, and this was repeated after 2min, making a total of 10 touches. The examiner performing the sensory testing was unaware of the animals' prior sensory testing results or the type of surgery performed on each animal, although there was no means of concealing postural abnormalities of the paw. On days in which both BP recording and sensory testing were performed, BP recordings were always done first to prevent any lasting effects of sensory testing on resting values of BP and HR. (A subset of animals had additional testing of other sensory modalities, which will be reported

elsewhere. Analysis by 2-way ANOVA showed that this testing did not affect the influence of injury upon the outcomes reported here.)

2.8 Statistical Analysis

We have previously showed that there is variability in pain behavior after SNL [14]. Specifically, while the majority of animals develop hyperalgesia after SNL, others do not despite being subjected to the same surgery. We therefore assigned SNL animals in this study to separate groups according to their type of response to pin stimulation. Those that showed a hyperalgesia-type response rate greater than 20% averaged over days 14 and 21 were included in the hyperalgesia (H) group, while the other animals who received SNL were included in the non-hyperalgesia (nonH) group. Data from these days were pooled for this categorization since there is day to day variation in pain behavior and post-injury pain behavior does not fully evolve until this time [14].

Not every animal in the data set for HR, MAP, and spontaneous baroreceptor sensitivity received a phenylephrine pressor test. For HR, MAP, spontaneous baroreceptor sensitivity, and behavior, data were included only from those animals that had data at every time point throughout the full time course of the experiment.

A two-way ANOVA with repeated measures design (the between factor was groups, the within factor was days; Statistica 8, StatSoft, Tulsa, OK) was used to determine if group membership affected baroreceptor sensitivity or resting levels of HR and MAP. If a main effect for group was significant, *post hoc* comparisons were performed in a way designed to limit the multiplicity of comparisons between groups across the various days. Specifically, planned comparisons were performed for particular time domains of interest as defined in prior research [15], including the baseline (day -1), the initial postoperative period (averaged over days 1–4) during which effects of acute operative injury may predominate, and the late period (averaged for days 16 and 20) when nerve injury-related hyperalgesia becomes established after SNL [14]. These comparisons, as well as the phenylephrine pressor test results, were analyzed by one-way ANOVA, and paired differences were evaluated using the Bonferroni test when a main effect of group was significant. Significance was set at $P < 0.05$. Data are reported as mean $\pm$ SEM.

3. Results

3.1 Grouping of subjects

Autopsy confirmed proper ligation and section in all SNL animals. Taking the SNL animals together, they showed a greater rate of hyperalgesia-type responses in pin testing averaged over days 14 and 21 ($39 \pm 32\%$, $n=18$) than the ShSNL animals ($2 \pm 5\%$; $P < 0.01$, $n=9$) or SI animals ($6 \pm 9\%$; $P < 0.01$, $n=8$). The rate of hyperalgesia-type responses to pin was not different between ShSNL and SI groups. Of the 18 SNL animals, 7 (39%) failed to develop hyperalgesia greater than 20% averaged over days 14 and 21 (nonH group), and showed an average of $5 \pm 2\%$ hyperalgesia-type responses, compared to $60 \pm 18\%$ hyperalgesia responses in the other SNL animals (H group; $P < 0.001$). This incidence of a failure to develop sustained hyperalgesia after SNL is very similar to that which occurs after SNL in the absence of contralateral femoral artery cannulation or transmitter implantation [14]. Using this grouping (H, nonH, SI, ShSNL; Fig. 2), there was a significant main effect of group averaged over days 1 and 3 after SNL ($P < 0.01$), and *post hoc* testing showed that the H group has a higher hyperalgesia rate than the SI group ($P < 0.05$).

Analysis of MAP, HR, baroreceptor sensitivity, and HRV showed no differences between ShSNL and SI groups (data not shown). Therefore, these were combined into a single control (C) group for all further analyses.

3.2 Cardiovascular parameters during rest

Analysis of MAP data (Fig. 3A) showed no differences between groups (ANOVA main effect $P=0.71$). However, analysis of HR (Fig. 3B) revealed a significant main effect of group ($P<0.001$). Secondary analysis for the baseline (day -1) showed a significant effect of group ($P<0.01$), with higher HR in animals that subsequently failed to become hyperalgesic (nonH group) in comparison to both the H group ($P<0.05$) and controls ($P<0.01$), but no difference between H and controls. To test whether preoperative (day -1) HR predicts the hyperalgesia-type response rate 14 to 21 days after SNL, we employed linear regression, which showed a significant, although weak, correlation in which low levels of hyperalgesia were associated with higher pre-injury HRs ($P<0.05$, $R^2=0.31$, $n=15$). Analysis of the initial 4 postoperative days showed a significant effect of group ($P<0.01$), with elevation of HR in nonH animals in comparison to both the H group ($P<0.01$) and controls ($P<0.01$), but no difference between H and controls. There was no significant correlation between HR on these days and hyperalgesia response rate 16 to 20 days after SNL ($P=0.11$). Analysis of HR over days 16 and 20 showed no significant effect of group ($P=0.14$).

3.3 Effect of nerve injury and pain on spontaneous baroreflex sensitivity

Analysis of baroreflex sensitivity across all days (Fig. 3C) showed a significant main effect of group ($P<0.05$). Secondary analysis for the baseline (day -1) showed no significant effect of group ($P=0.40$). Analysis of the initial 4 postoperative days, however, showed a significant effect of group ($P<0.05$), in which nonH baroreceptor sensitivity was elevated in comparison to both the H group ($P<0.01$) and controls ($P<0.05$). Analysis of baroreceptor sensitivity over days 16 and 20 showed a significant effect of group ($P<0.05$) and a decrease in the H group compared to controls ($P<0.05$) and nonH ($P<0.05$). Regression between spontaneous baroreflex sensitivity and the degree of hyperalgesia after SNL was not significant for baroreflex on days 1 to 4 ($P=0.06$) and days 16 to 20 ($P=0.29$).

3.4 Effect of nerve injury and pain on phenylephrine pressor test

The phenylephrine pressor test was performed as a terminal experiment on day 22 to determine baroreflex sensitivity in the anesthetized rats. Setpoint levels during anesthesia prior to administration of phenylephrine showed no effects of injury on MAP (106 ± 1 mmHg in C, 105 ± 14 mmHg in nonH, 104 ± 7 in H; $P=0.88$) or HR (355 ± 23 in C, 373 ± 23 in nonH, 345 ± 31 in H; $P=0.16$). Analysis of baroreceptor sensitivity confirmed the findings of the spontaneous method (Fig. 4) and identified a main effect of group ($P<0.05$), in which *post hoc* paired comparisons showed baroreceptor sensitivity of animals in the H group was significantly lower compared to the C group ($P<0.05$) and the nonH group ($P<0.05$). Measurements with the phenylephrine method showed a lower sensitivity in each group compared to the spontaneous baroreflex sensitivity in conscious animals, which is generally true in comparing values from pharmacological and spontaneous techniques [28], and may also reflect suppression of the baroreceptor by our use of general anesthesia during measurement of baroreceptor reflex gain with phenylephrine [32].

3.5 Heart rate variability analysis

HF power, which reflects vagal control of resting HR, was influenced by group (Fig. 5A; ANOVA main effect for group $P<0.05$; for interaction of group and day $P<0.01$), with lower HF power in the nonH group at day -1 ($P<0.05$ vs. C) and days 1 and 4 combined ($P<0.01$ vs. C, $P<0.05$ vs. H), and no difference between the C and H groups. Similarly, the LF/HF

ratio, which reflects sympatho-vagal balance, was also influenced by group (Fig. 5B; ANOVA main effect for injury group $P<0.01$, for interaction of group and day $P<0.001$), with significant elevation in the nonH group at day -1 ($P<0.001$ vs. C, $P<0.01$ vs. H) and days 1 and 4 combined ($P<0.01$ vs. C, $P<0.05$ vs. H), and no difference between the C and H groups. There were no differences between groups at the late time points.

4. Discussion

Homeostasis in the circulatory system is dependent upon neural regulation driven by central nervous system sites including the nucleus tractus solitarius (NTS), where a variety of inputs from cardiovascular, pulmonary and somatic sensory systems are integrated to drive efferent signals that control vascular tone and cardiac function. Numerous prior observations indicate that the somatic sensory system and circulatory regulation are closely linked. Intense painful stimulation is typically accompanied by circulatory activation manifest as increases in BP and HR. Reciprocally, it is well established that both chronic and acute elevations in BP produce a relative insensitivity to brief, phasic pain [40], although sensitivity to sustained inflammatory stimulation is amplified [38,39]. For both of these phenomena, the mechanism is unknown. Dysfunction of the autonomic nervous system also accompanies chronic painful conditions such as nerve injury [24,30] and chronic regional pain syndrome (CRPS) [2]. The present experiments reveal an additional link between circulatory and sensory control by identifying an influence of chronic pain upon the baroreflex pathway.

The baroreflex system serves to buffer changes in BP by activating vagal efferent activity and reducing sympathetic activity when BP increases, and *vice versa*, resulting in compensatory adjustments of HR, cardiac contractility, peripheral vascular resistance and venous return. Our data show that the sensitivity of this feedback system is diminished following peripheral nerve injury by SNL. A critical finding is that baroreceptor reflex compromise is selective for only those animals that develop hyperalgesia-type behavior (H group) in response to nociceptive stimulation. Both the spontaneous and the phenylephrine infusion methods reveal depressed baroreceptor sensitivity in animals that show sustained hyperalgesic behavior after SNL. In contrast, baroreceptor function is unaffected in control animals (SI or sham SNL surgery) as well as those that have nerve injury but do not develop sustained hyperalgesia (nonH group). Thus, the decreased baroreceptor sensitivity in the H group cannot be attributed to SNL *per se*, nor to the consequences of extensive paravertebral surgery. The early postoperative occurrence of hyperalgesia after sham SNL is not typical [14] and may be due to the additional influences of contralateral injury with cannulation or to transmitter implantation. Although care was taken to avoid injury to the femoral nerve, it lies immediately adjacent to the femoral artery and may have been irritated by the presence of the chronic catheter. Actions of this irritation that act globally or specifically on contralateral pain processing [18] may also explain the occasional hyperalgesia-type responses prior to the animals' second surgery and after skin incision or sham SNL. In sham SNL and nonH groups, hyperalgesia dissipates within 2 weeks, which indicates a different process than that causing persistent hyperalgesia in the H group. The absence of baroreceptor suppression during this early phase of nonspecific hyperalgesia in sham SNL, nonH animals supports a specific connection of baroreflex suppression to hyperalgesia caused specifically by nerve injury. Our finding that early hyperalgesia in the H group is not accompanied by baroreceptor suppression suggests that hyperalgesia must be sustained beyond 2 weeks to affect reflex function. It is known that mechanisms supporting pain early after axotomy, including neurotrophin withdrawal, differ from later mechanisms such as neuronal loss [9].

Another study found relative bradycardia after sciatic nerve injury [15], which was attributed to enhanced parasympathetic modulation of HR from increased baroreflex sensitivity, in contrast to our findings. However, baroreflex function was not directly tested in that study.

Also, the chronic constriction injury model used in that study differs from SNL in being more distal, and includes an important component of perineural inflammation [22]. Additionally, their use of older rats, and even intraperitoneal transmitter implantation and jugular cannulation, may have contributed to their different findings.

Acute nociceptive stimulation in rats by application of a clamp to the paw for 10s diminishes baroreceptor sensitivity [7]. This has been proposed to occur at the level of second order baroreceptive neurons in the NTS that directly receive baroreceptor afferent input [8]. Nociceptive inputs release substance P in the NTS, which excites GABAergic interneurons, leading to release of GABA and subsequent depression of the baroreceptive neurons in the NTS. Since long term baroreceptor plasticity has been demonstrated during experimental hypertension [11] with reversal over 3 days [16], it is possible that the shift in baroreceptor function in the present study resulted from the cumulative effects of repeated pain events in the hyperalgesic rats, leading to a sustained resetting of baroreflex sensitivity. Also, it has been shown that spontaneously hypertensive rats, which have depressed baroreflex function [13, 25], show exaggerated pain behavior to sustained noxious stimulation from inflammation [38,39]. While a causal interaction has not been established, this association suggests that baroreceptor suppression may be linked to chronic pain in settings other than nerve injury, and that baroreflex suppression may be a contributing factor hyperalgesia. From our current observations, however, it is clear that baroreceptor suppression alone cannot explain the early hyperalgesia seen in sham SNL, H, and nonH groups.

Despite the development of diminished baroreceptor sensitivity in the H group at later time points, our findings show no concurrent HR or MAP alterations. Consistent with this were the normal HRV indices and baroreceptor setpoints at the same time period. Observations in other conditions have shown that changes in baroreflex sensitivity need not be accompanied by changes in resting BP or HR. For instance, studies have found a decrease in baroreceptor sensitivity in such conditions as orthostatic intolerance/hypotension [31], aging [33], and familial dysautonomia [37], in which there has been no change in HR or BP.

As in human disease, there is variability in the development of sensory changes in rats despite a stereotyped nerve injury by SNL [14]. In the present study, animals that fail to develop hyperalgesia show several distinctive cardiovascular features prior to injury. Specifically, these nonH animals had a higher resting HR prior to SNL. Consistent with this, HRV analysis shows elevated HF power and depression of LF/HF ratio in these same animals, indicative of a shift in their baseline autonomic balance towards decreased vagal and increased sympathetic regulation, which may be an influence that imparts resistance to neuropathic pain behavior. These animals also showed an amplification of baroreceptor sensitivity immediately following surgery. This shift in cardiovascular regulation was not seen in either H animals, nor in control animals that had sham SNL or only skin incision. Therefore, it cannot be explained by the lack of hyperalgesia *per se* as this was also true in the control groups. Further, the elevated HR and distinct HRV parameters predated the nerve injury surgery, so they cannot be attributed to factors that might regulate development of hyperalgesia such as spinal nerve anatomy or sensitivity of cellular mechanisms to nerve injury. Although the same transmitter implantation and arterial cannulation surgery was also performed in all groups including those that did not develop elevated HR or autonomic changes, it is possible that particularly high surgical stress from implantation surgery may have contributed to these cardiovascular features and pain resistance. Evidently, animals that subsequently fail to develop hyperalgesia have preexisting differences in cardiovascular regulation and a distinctive response to surgical stress that in some fashion provide intrinsic resistance to developing hyperalgesia after nerve injury, or are associated with other factors that have this effect. Consistent with this view, it is recognized that baroreceptor activation from a variety of causes results in hypoalgesia [40]. We cannot eliminate the alternative possibility that amplified baroreceptor activity may in some fashion

limit sympathetic contributions to pain generation in this group of animals. In contrast to our HRV findings, other reports suggest an analgesic effect of vagal activation [17], and our findings should be confirmed with additional prospective trials.

In humans, diminished baroreceptor function permits greater fluctuation of BP and excessive sympathetic activity, which may produce dysrhythmias, myocardial ischemia, and vascular and end organ damage [20]. Substantial evidence indicates that depressed baroreceptor sensitivity is associated with high medical risk. Loss of baroreflex sensitivity independently predicts a strongly increased probability of sudden cardiac death following myocardial infarction, due to sympathetic hyperactivity and loss of compensatory vagal activity [19]. Baroreflex loss may also contribute to complications of hypertension, heart failure, and stroke [20]. Other conditions that decrease baroreflex sensitivity include prolonged bed rest and advanced age. It is possible that baroreceptor sensitivity may be diminished in human subjects suffering from chronic neuropathic pain as we have shown in an animal model, in which case there is a possibility that this may result in a higher risk for adverse cardiovascular outcomes. If this speculation were to prove correct, there is a potential that specific therapy to improve baroreflex sensitivity [3,4] might prolong survival of these patients.

Acknowledgments

Funding was provided by grant NS-42150 to Q.H. from the National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland, USA; VA Medical Research Funds to J.S.; and National Science Foundation Grant IOS 0751613 to C.D.

References

1. Abram SE, Kostreva DR, Hopp FA, Kampine JP. Cardiovascular responses to noxious radiant heat in anesthetized cats. *Am J Physiol* 1983;245:R576–R580. [PubMed: 6624953]
2. Baron R, Maier C. Reflex sympathetic dystrophy: skin blood flow, sympathetic vasoconstrictor reflexes and pain before and after surgical sympathectomy. *Pain* 1996;67:317–326. [PubMed: 8951925]
3. Bechir M, Enseleit F, Chenevard R, Luscher TF, Noll G. Effect of losartan on muscle sympathetic activity and baroreceptor function in systemic hypertension. *Am J Cardiol* 2005;95:129–131. [PubMed: 15619411]
4. Bechir M, Enseleit F, Chenevard R, Muntwyler J, Luscher TF, Noll G. Folic Acid improves baroreceptor sensitivity in hypertension. *J Cardiovasc Pharmacol* 2005;45:44–48. [PubMed: 15613978]
5. Bertinieri G, di Rienzo M, Cavallazzi A, Ferrari AU, Pedotti A, Mancia G. A new approach to analysis of the arterial baroreflex. *J Hypertens Suppl* 1985;3:S79–S81. [PubMed: 2856787]
6. Bertinieri G, Di Rienzo M, Cavallazzi A, Ferrari AU, Pedotti A, Mancia G. Evaluation of baroreceptor reflex by blood pressure monitoring in unanesthetized cats. *Am J Physiol* 1988;254:H377–H383. [PubMed: 3344828]
7. Boscan P, Kasparov S, Paton JF. Somatic nociception activates NK1 receptors in the nucleus tractus solitarius to attenuate the baroreceptor cardiac reflex. *Eur J Neurosci* 2002;16:907–920. [PubMed: 12372027]
8. Boscan P, Pickering AE, Paton JF. The nucleus of the solitary tract: an integrating station for nociceptive and cardiorespiratory afferents. *Exp Physiol* 2002;87:259–266. [PubMed: 11856972]
9. Devor, M. Response of nerves to injury in relation to neuropathic pain. In: McMahon, S.; Koltzenburg, M., editors. *Wall and Melzack's textbook of pain*. London: Churchill Livingstone; 2006. p. 905-927.
10. Eckberg DL. Sympathovagal balance: a critical appraisal. *Circulation* 1997;96:3224–3232. [PubMed: 9386196]
11. Floras JS, Hassan MO, Jones JV, Osikowska BA, Sever PS, Sleight P. Consequences of impaired arterial baroreflexes in essential hypertension: effects on pressor responses, plasma noradrenaline and blood pressure variability. *J Hypertens* 1988;6:525–535. [PubMed: 3171169]

12. Furlan R, Colombo S, Perego F, Atzeni F, Diana A, Barbic F, Porta A, Pace F, Malliani A, Sarzi-Puttini P. Abnormalities of cardiovascular neural control and reduced orthostatic tolerance in patients with primary fibromyalgia. *J Rheumatol* 2005;32:1787–1793. [PubMed: 16142879]
13. Head GA, Adams MA. Time course of changes in baroreceptor reflex control of heart rate in conscious SHR and WKY: contribution of the cardiac vagus and sympathetic nerves. *Clin Exp Pharmacol Physiol* 1988;15:289–292. [PubMed: 3271610]
14. Hogan Q, Sapunar D, Modric-Jednacak K, McCallum JB. Detection of neuropathic pain in a rat model of peripheral nerve injury. *Anesthesiology* 2004;101:476–487. [PubMed: 15277932]
15. Jin Y, Sato J, Yamazaki M, Omura S, Funakubo M, Senoo S, Aoyama M, Mizumura K. Changes in cardiovascular parameters and plasma norepinephrine level in rats after chronic constriction injury on the sciatic nerve. *Pain* 2008;135:221–231. [PubMed: 17611035]
16. Jones JV, Floras JS. Baroreflex sensitivity changes during the reversal of Goldblatt two-kidney one-clip hypertension in rats. *Clin Exp Hypertens A* 1982;4:903–914. [PubMed: 7094365]
17. Kirchner A, Stefan H, Bastian K, Birklein F. Vagus nerve stimulation suppresses pain but has limited effects on neurogenic inflammation in humans. *Eur J Pain* 2006;10:449–455. [PubMed: 16125425]
18. Koltzenburg M, Wall PD, McMahon SB. Does the right side know what the left is doing? *Trends in Neurosciences* 1999;22:122–127. [PubMed: 10199637]
19. La Rovere MT, Bigger JT Jr, Marcus FI, Mortara A, Schwartz PJ. ATRAMI (Autonomic Tone and Reflexes After Myocardial Infarction) Investigators. Baroreflex sensitivity and heart-rate variability in prediction of total cardiac mortality after myocardial infarction. *Lancet* 1998;351:478–484. [PubMed: 9482439]
20. Lanfranchi PA, Somers VK. Arterial baroreflex function and cardiovascular variability: interactions and implications. *Am J Physiol Regul Integr Comp Physiol* 2002;283:R815–R826. [PubMed: 12228049]
21. Malliani A, Pagani M, Lombardi F, Cerutti S. Cardiovascular neural regulation explored in the frequency domain. *Circulation* 1991;84:482–492. [PubMed: 1860193]
22. Maves TJ, Pechman PS, Gebhart GF, Meller ST. Possible chemical contribution from chronic gut sutures produces disorders of pain sensation like those seen in man. *Pain* 1993;54:57–69. [published erratum appears in *Pain* 1993 Oct;55(1):131–4]. [PubMed: 8378104]
23. Meier PM, Alexander ME, Sethna NF, De Jong-De Vos Van Steenwijk CC, Zurakowski D, Berde CB. Complex regional pain syndromes in children and adolescents: regional and systemic signs and symptoms and hemodynamic response to tilt table testing. *Clin J Pain* 2006;22:399–406. [PubMed: 16691095]
24. Michaelis M, Devor M, Janig W. Sympathetic modulation of activity in rat dorsal root ganglion neurons changes over time following peripheral nerve injury. *Journal of Neurophysiology* 1996;76:753–763. [PubMed: 8871196]
25. Minami N, Head GA. Relationship between cardiovascular hypertrophy and cardiac baroreflex function in spontaneously hypertensive and stroke-prone rats. *J Hypertens* 1993;11:523–533. [PubMed: 8390524]
26. Oosting J, Struijker-Boudier HA, Janssen BJ. Validation of a continuous baroreceptor reflex sensitivity index calculated from spontaneous fluctuations of blood pressure and pulse interval in rats. *J Hypertens* 1997;15:391–399. [PubMed: 9211174]
27. Pickering AE, Boscan P, Paton JF. Nociception attenuates parasympathetic but not sympathetic baroreflex via NK1 receptors in the rat nucleus tractus solitarius. *J Physiol* 2003;551:589–599. [PubMed: 12813142]
28. Rudas L, Crossman AA, Morillo CA, Halliwill JR, Tahvanainen KU, Kuusela TA, Eckberg DL. Human sympathetic and vagal baroreflex responses to sequential nitroprusside and phenylephrine. *Am J Physiol* 1999;276:H1691–H1698. [PubMed: 10330255]
29. Sato A, Sato Y, Schmidt RF. The impact of somatosensory input on autonomic functions. *Rev Physiol Biochem Pharmacol* 1997;130:1–328. [PubMed: 9019119]
30. Sato J, Perl ER. Adrenergic excitation of cutaneous pain receptors induced by peripheral nerve injury. *Science* 1991;251:1608–1610. [PubMed: 2011742]
31. Schrezenmaier C, Singer W, Swift NM, Sletten D, Tanabe J, Low PA. Adrenergic and vagal baroreflex sensitivity in autonomic failure. *Arch Neurol* 2007;64:381–386. [PubMed: 17353381]

32. Seagard JL, Elegbe EO, Hopp FA, Bosnjak ZJ, von Colditz JH, Kalbfleisch JH, Kampine JP. Effects of isoflurane on the baroreceptor reflex. *Anesthesiology* 1983;59:511–520. [PubMed: 6650907]
33. Shi X, Huang G, Smith SA, Zhang R, Formes KJ. Aging and arterial blood pressure variability during orthostatic challenge. *Gerontology* 2003;49:279–286. [PubMed: 12920347]
34. Smyth HS, Sleight P, Pickering GW. Reflex regulation of arterial pressure during sleep in man. A quantitative method of assessing baroreflex sensitivity. *Circ Res* 1969;24:109–121. [PubMed: 4303309]
35. Stauss HM, Morgan DA, Anderson KE, Massett MP, Kregel KC. Modulation of baroreflex sensitivity and spectral power of blood pressure by heat stress and aging. *Am J Physiol* 1997;272:H776–H784. [PubMed: 9124438]
36. Stauss HM, Persson PB. Cardiovascular variability and the autonomic nervous system. *J Hypertens* 2006;24:1902–1905. [PubMed: 16915045]
37. Stemper B, Bernardi L, Axelrod FB, Welsch G, Passino C, Hilz MJ. Sympathetic and parasympathetic baroreflex dysfunction in familial dysautonomia. *Neurology* 2004;63:1427–1431. [PubMed: 15505160]
38. Taylor BK, Peterson MA, Basbaum AI. Exaggerated cardiovascular and behavioral nociceptive responses to subcutaneous formalin in the spontaneously hypertensive rat. *Neurosci Lett* 1995;201:9–12. [PubMed: 8830322]
39. Taylor BK, Roderick RE, St Lezin E, Basbaum AI. Hypoalgesia and hyperalgesia with inherited hypertension in the rat. *Am J Physiol Regul Integr Comp Physiol* 2001;280:R345–R354. [PubMed: 11208561]
40. Zamir N, Maixner W. The relationship between cardiovascular and pain regulatory systems. *Ann N Y Acad Sci* 1986;467:371–384. [PubMed: 3524385]

Figure 1.

Method for determining spontaneous baroreceptor sensitivity. A. Sequences of heart beats in the telemetrically recorded blood pressure (BP) trace are sought that show sequential rise of blood pressure for a series of beats (up-sequence) or sequential fall (down-sequence, not shown). The systolic blood pressure for the beats in the sequence (arrows) are paired with the inter-beat interval (ibi) that follows four beats later. This time delay compensates for the physiological delay in the baroreceptor reflex [26]. B. Regression between the instantaneous heart rate, calculated from the ibi, and the blood pressure for each beat is fit with a straight line, resulting in a slope that corresponds to the spontaneous baroreceptor sensitivity for that sequence. The upper line (diamonds) is from the data shown in A., and is from a rat that shows

hyperalgesia behavior 16 days following spinal nerve ligation injury. The lower line (squares) shows similar data from a control animal 16 days after SNL surgery. R^2 is the square of the correlation coefficient for the points in that line.

Figure 2.

Sensory evaluation of rats by punctate mechanical nociceptive stimulation with a pin in the various study groups. The percentage of hyperalgesia-type behavior (sustained lifting, shaking, chewing or grooming of the paw and toes) in 10 touches of the right foot is shown at baseline (day -1) and after skin incision only (n = 8), right sided sham spinal nerve ligation (Sham SNL; n = 9), and right sided SNL. The SNL animals are further divided into groups that developed greater than 20% hyperalgesia-type responses averaged over days 14 and 21 (Hyperalgesic SNL, n = 11), and those that did not (Non-Hyperalgesic SNL, n = 7). * indicates a significant difference between Hyperalgesic SNL and Skin Incision groups averaged over days 1 and 3. § indicates a significant difference between Hyperalgesic SNL and all other groups averaged over days 14 and 21.

Figure 3.

Cardiovascular parameters measured by telemetry in rats before (day -1) and after sham spinal nerve ligation or skin incision (combined as Control group, $n=21$). The other groups consist of animals that developed hyperalgesia behavior after spinal nerve ligation (Hyperalgesic group, $n=8$), and animals that also received spinal nerve ligation but did not develop hyperalgesia behavior (Non-Hyperalgesic group, $n=7$). A. Mean arterial pressure showed no main effect of group by ANOVA. There is an age related increase in mean arterial pressure over time. B. Heart rate showed a significant main effect of group ($P < 0.001$). Secondary analysis for day -1 showed a significant effect of group ($P < 0.01$, §), with higher heart rate in the Non-Hyperalgesic group in comparison to both the Hyperalgesic group ($P < 0.05$) and

Controls ($P<0.01$). Analysis of the initial 4 postoperative days showed a significant effect of group ($P<0.01$, §), with elevation of heart rate in Non-Hyperalgesic animals in comparison to both the Hyperalgesic group ($P<0.01$) and Controls ($P<0.01$). C. Baroreflex sensitivity determined by the spontaneous method showed a significant main effect of group ($P<0.05$). Analysis of the initial 4 postoperative days showed a significant effect of group ($P<0.05$, *), with elevation of baroreflex sensitivity in Non-Hyperalgesic animals in comparison to both the Hyperalgesic group ($P<0.01$) and Controls ($P<0.05$). Sensitivity over days 16 and 20 also showed a significant main effect of group ($P<0.05$, *) with a decrease in the Hyperalgesic group compared to both the Non-Hyperalgesic group ($P<0.05$) and Controls ($P<0.05$).

Figure 4.

Baroreflex sensitivity determined by phenylephrine test. There was a significant effect of group on baroreflex sensitivity ($P<0.05$). Paired *post hoc* comparisons showed that animals in the Hyperalgesic group, which are those that developed hyperalgesia after spinal nerve ligation, had a significantly lower baroreflex gain on day 22 after surgery compared to both the Control group, comprised of sham SNL and skin incision animals ($P<0.05$), and to the Non-Hyperalgesic group, comprised of animals that did not develop hyperalgesia after spinal nerve ligation ($P<0.05$). Brackets indicate significant differences, and the numbers in the bars indicate the number of animals in each group.

Figure 5.

Power spectral analysis of baseline heart rate (HR). A. In the non-hyperalgesic group of rats that failed to develop sustained hyperalgesia after spinal nerve ligation (SNL), high frequency (HF) power as a percent of total power was lower before SNL (day -1, $P < 0.05$ vs. controls, *) and initially after SNL at days 1 and 4 combined ($P < 0.01$ vs. controls, $P < 0.05$ vs. hyperalgesic animals, §). B. The ratio of low frequency (LF) to HF power was higher in the non-hyperalgesic group at day -1 ($P < 0.001$ vs. controls, $P < 0.01$ vs. hyperalgesic animals, §), and at days 1 and 4 combined ($P < 0.01$ vs. controls, $P < 0.05$ vs. hyperalgesic animals, *).