**Thiamine Suppresses Thermal Hyperalgesia, Inhibits Hyperexcitability, and Lessens Alterations of Sodium Currents in Injured, Dorsal Root Ganglion Neurons in Rats**

Xue-Song Song, M.D., Ph.D., Zhi-Jiang Huang, Ph.D., † Xue-Jun Song, M.D., Ph.D.

**Background:** B vitamins can effectively attenuate inflammatory and neuropathic pain in experimental animals, while their efficacy in treating clinical pain syndromes remains unclear. To understand possible mechanisms underlying B vitamin-induced analgesia and provide further evidence that may support the clinical utility of B vitamins in chronic pain treatment, this study investigated effects of thiamine (B1) on the excitability and Na\(^+\) currents of dorsal root ganglion (DRG) neurons that have been altered by nerve injury.

**Methods:** Nerve injury was mimicked by chronic compression of DRG in rats. Neuropathic pain was evidenced by the presence of thermal hyperalgesia. Intracellular and patch-clamp recordings were made *in vitro* from intact and dissociated DRG neurons, respectively.

**Results:** (1) *In vivo* intraperitoneal administration of B1 (66 mg/kg/day, 10–14 doses) significantly inhibited DRG compression-induced neural hyperexcitability, in addition to suppressing thermal hyperalgesia. (2) *In vitro* perfusion of B1 (0.1, 1 and 10 μM) resulted in a dose-dependent inhibition of DRG neuron hyperexcitability. In addition, the DRG neurons exhibited size-dependent sensitivity to B1 treatment, i.e., the small and the medium-sized neurons, compared to the large neurons, were significantly more sensitive. (3) Both *in vitro* (1 μM) and *in vivo* application of B1 significantly reversed DRG compression-induced down-regulation of tetrodotoxin-resistant but not tetrodotoxin-sensitive Na\(^+\) current density in the small neurons. B1 at 1 μM also reversed the compression-induced hyperpolarizing shift of the inactivation curve of the tetrodotoxin-resistant currents and the upregulated ramp currents in small DRG neurons.

**Conclusion:** Thiamine can reduce hyperexcitability and lessen alterations of Na\(^+\) currents in injured DRG neurons, in addition to suppressing thermal hyperalgesia.

The B vitamins thiamine (B1), pyridoxine (B6), and cyanocobalamin (B12) have been demonstrated to be clinically useful in treating disorders such as diabetic polyneuropathy\(^1\)–\(^3\) and rheumatoid arthritis\(^4\) in addition to suppressing thermal hyperalgesia. 2,5,6 Several lines of study have provided strong evidence that these B vitamins have analgesic and antiinflammatory capacities in experimental animals. 6–11 Recent studies have further demonstrated that these B vitamins can effectively attenuate neuropathic pain syndromes in animals with peripheral nerve injury, dorsal root ganglion (DRG) compression (CCD)\(^12,13\) or diabetes.\(^14\) These studies indicate a novel effect of the B vitamins on neuropathic pain and suggest the possible clinical utility of B vitamins for treating similar neuropathies. However, the neural mechanisms underlying B vitamin-induced inhibition of neuropathic pain remain unknown and there is a lack of clear evidence to support the efficacy of B vitamins in treating clinical pain syndromes.\(^1\)–\(^4\),\(^15\)

Injury to the peripheral axons and/or somata of DRG neurons can cause severe hyperalgesia and allodynia associated with neural hyperexcitability. 2,\(^16\)–\(^23\) The DRG neuron hyperexcitability is thought to underlie neuropathic pain by causing central sensitization. Voltage-gated sodium channels (VGSCs), which are necessary for electrogensis and nerve impulse conduction, can be dynamically regulated after nerve injury, DRG compression, or peripheral inflammation and play important roles in modulating neural excitability.\(^16\)\(^,24\)–\(^26\) In DRG neurons these VGSCs include Nav1.8 and Nav1.9, which conduct tetrodotoxin-resistant (TTX-R) currents, and Nav1.3, Nav1.6, and Nav1.7, which conduct tetrodotoxin-sensitive (TTX-S) currents. Several reports have shown that TTX-R currents, Nav1.8 mRNA, and Nav1.8 protein significantly decrease in nociceptive DRG neurons after nerve injury in animals\(^16,25\) as well as in patients.\(^27\) The TTX-S currents and channels are also altered in models of nerve injury or inflammation,\(^25\) and down-regulation of Nav1.7 appears to play an important role in neuropathic pain sensation in erythromelalgia.\(^28\) Conversely, interference with Nav1.8 expression can suppress neuropathic pain.\(^29\)–\(^32\) Interestingly, some anticonvulsants useful in neuropathic pain might work through sodium channel modulation.

Therefore, we hypothesized that the B vitamins might relieve thermal hyperalgesia by suppressing neural hyperexcitability and modulating altered Na\(^+\) currents in injured DRG neurons. Our results provide the first evidence that vitamin B1 can reduce neural hyperexcitability and modulate TTX-R and TTX-S Na\(^+\) currents of DRG neurons, in addition to suppressing thermal hyperalgesia in rats with CCD treatment, suggesting that B1-induced inhibition of neural hyperexcitability in injured DRG neurons may contribute to B1-induced suppression of...
thermal hyperalgesia in rats given CCD treatment. At present, the effects of B1 on Na⁺ currents in injured DRG neurons have an uncertain relationship to the effects of B1 on neuropathic pain expressed behaviorally. This study provides further evidence supporting the clinical utility of B vitamins in chronic pain treatment. Preliminary data from this study have been published in abstract form.

Materials And Methods

Animals and Surgical Procedures

Experimental procedures were performed on adult, male Sprague-Dawley rats (180–250 g, n = 129) and were conducted in accordance with the regulations of the ethics committee of the International Association for the Study of Pain and approved by the Parker Research Institute Animal Care and Use Committee (Dallas, Texas). All surgeries were done under anesthesia induced by intraperitoneal administration of sodium pentobarbital (50 mg/kg). Hollow stainless-steel, L-shaped rods (4 mm in length and 0.6 mm in diameter) were surgically implanted unilaterally into the intervertebral foramen (IVF) at L4 and L5 to chronically compress the DRG (CCD treatment). In brief, rats (n = 55) were anesthetized, paraspinal muscles were separated from the mammillary and transverse processes, and the IVF of L4 and L5 was exposed. A rod was implanted chronically into the IVF. In separate animals (n = 32), the rod implanted into the IVF was connected to silicon tubing to permit delivery of solution to the DRG during compression. The method was similar to that which we have recently described. After surgery, the muscle and skin layers were sutured. Sham surgery (n = 42) involved surgical procedures identical to those described but without insertion of the rod.

Behavioral Testing

Thermal hyperalgesia was indicated by a decrease in the latency of foot withdrawal evoked by a radiant heat stimulus generated and controlled by a IITC Model 356 Analgesia Meter (Life Science, Series 8, Woodland Hills, CA). The protocol was the same as that which we have described previously. The rats were tested on each of 2 successive days prior to surgery. Postoperative tests were conducted 1, 3, and 5 days after surgery and/or on the day of electrophysiological recording (days 10–14). Thermal hyperalgesia for a given rat was defined as a postsurgical decrease of foot withdrawal latency from the mean preoperative value, with a difference score ≥ 3 s. Significant B1-induced inhibition of thermal hyperalgesia for a given rat was defined as a decrease in the difference in foot withdrawal latency from the mean preoperative value, with a difference score ≤ 2.5 s. Only rats that exhibited thermal hyperalgesia after CCD treatment or significant B1-induced inhibition of hyperalgesia were used for the electrophysiological studies.

Excised, Intact DRG Neuron Preparation

DRG neurons were tested while still in place in excised ganglia prepared as described previously, using L₄ and/or L₅ ganglia in rats that previously received CCD (n = 35) or sham surgery (n = 16). Briefly, a laminectomy was performed under anesthesia. Ice-cold, oxygenated, buffered solution containing (in mM) NaCl, 3.5 KCl, 1.5 CaCl₂, 1 MgCl₂, 4.5 HEPES, 5.5 HEPES-Na, and 10 glucose (pH 7.3, osmolarity 310–320 mOsm) was dripped onto the surface of the ganglion during the procedure. The ganglia from the left side of the L₄ and L₅ segments were removed and placed in 35-mm petri dishes containing ice-cold, oxygenated, buffered solution. The perineurium and epineurium were peeled off and the attached sciatic nerve and dorsal roots transected adjacent to the ganglion. The otherwise intact ganglion was then treated with collagenase (type P, 1 mg/ml, Boehringer Mannheim GmbH, Mannheim, Germany) for 30 min at 35°C, transferred to the recording chamber, and mounted on the stage of an upright microscope (BX50-WT, Olympus, Tokyo, Japan). A U-shaped stainless steel rod with four crossing silver wires held the ganglion gently in place. The DRG was incubated in the oxygenated, buffered solution at room temperature (21–22°C).

Dissociation Of DRG Neurons

DRG neurons were dissociated from L₄ and/or L₅ ganglia taken from rats that previously received CCD (n = 20) or sham surgery (n = 18). The protocol was the same as that which we have described recently. In brief, the excised ganglion was minced using microdissection scissors, the DRG fragments transferred into 10 ml of the buffered solution containing collagenase (type IA, 1 mg/ml, Sigma, St. Louis, MO) and trypsin (0.5 mg/ml, Sigma), and then incubated for 30 min at 35°C. The DRG fragments were removed, rinsed 5–6 times in the buffered solution, and put into the buffered solution (5 ml) containing DNase (0.2 mg/ml, Sigma) to prevent possible toxicity from DNA leaking from ruptured cells. Individual neurons were dissociated by passing DRG fragments through a set of fire-polished glass pipettes with decreasing diameter.

Intracellular Recordings

Intracellular recordings were made from the intact DRG somata 2–6 h after dissociation using conventional bridge-balance techniques (Axoclamp-2B, Axon Instruments, Foster City, CA) and analyzed with pCLAMP-8 (Axon Instruments). Somata of the DRG neurons were classified visually by their diameters as small (≤ 30 μm), medium (31–49 μm), or large (≥ 50 μm). Glass microelectrodes were fabricated with a Flaming/Brown mi-
cropipette puller (Model P-97/PC, Sutter Instruments, Novato, CA) and filled with 2M potassium acetate (pH = 7.2). The protocols used to record and measure the neural electrophysiological properties were similar to those we have described recently.\(^{19,22}\) Resting membrane potential (RMP) was taken 2–3 min after RMP had stabilized. All neurons accepted for testing exhibited a stable RMP of \(-45 \text{ mV}\) or more negative. Action potential (AP) current threshold was defined as the minimum current required for evoking an AP. Repetitive discharge of each neuron was measured by counting the spikes evoked by intracellular injection of standardized depolarizing currents at 2.5 \(\times\) threshold strength (\( \times 1000 \text{ ms} \)).

**Whole Cell Patch-clamp Recordings**

Standard whole-cell patch-clamp recordings were conducted in small DRG neurons at room temperature (21–22°C) 2–8 h after dissociation. Fire-polished electrodes had a resistance of 1–3 MΩ. The pipette solution contained (in mM): 110 CsF, 11 EGTA, 10 NaCl, MgCl\(_2\) 5, and 10 HEPES, pH 7.3 with CsOH. The bath solution contained (in mM): 65 NaCl, 2.5 KCl, 5 MgCl\(_2\), 0.01 CaCl\(_2\), 50 Choline-Cl, 20 TEA-Cl, 5 glucose, 5 Na-HEPES, and 5 HEPES, pH 7.4 with NaOH. Voltage-clamped currents were recorded with an Axopatch-200B amplifier (Molecular Devices, Union City, CA) and data acquired and analyzed with the pCLAMP v10.0 software (Molecular Devices) filtered with a low-pass Bessel filter setting of 5 kHz and digitized at a sampling rate of 40 kHz. The membrane capacitance (\(C_m\)) was read from the amplifier. Voltage errors were minimized by using 80–90% series resistance compensation, and the capacitance artifact was canceled by the patch-clamp amplifier. Linear leakage currents were digitally subtracted on-line using a P/6 procedure. Data acquisition began 5 min after establishing whole-cell configuration, and the holding potential was \(-80 \text{ mV}\).

Somata of the small DRG neurons were classified by their diameters (15–30 μm) and \(C_m\) (\(\leq 45 \text{ pF}\)). Neurons were not considered for analysis if they had high leakage currents (holding current > 1.0 nA at \(-80 \text{ mV}\)), membrane blebs, total sodium current < 500 pA, or an access resistance > 5 MΩ. Access resistance was monitored throughout the experiment, and data were not used if resistance changes of > 20% occurred. Data were not corrected for liquid junction potentials. The offset potential was zeroed before patching the cells and checked after each recording for drift.

To analyze the voltage dependence of channel activation, the sodium conductance (G) was calculated. Peak current data for each cell were divided by the respective driving force (\(V_m - V_{rev}\)), plotted against \(V_m\), and fit to a Boltzmann distribution equation of the following form:

\[
G/G_{max} = 1/(1 + \exp ((V_{1/2} - V_m)/k))
\]

where \(G_{max}\) is the maximum G, \(V_{1/2}\) is the potential at which activation is half-maximal, and \(k\) is the slope of the curve.

For the analysis of steady-state fast inactivation kinetics, the inactivation parameter was fitted to a Boltzmann distribution equation:

\[
I/I_{max} = 1/(1 + \exp ((V_{1/2} - V_{rev})/k)),
\]

where \(I_{max}\) is the maximum sodium current elicited after the most hyperpolarized prepulse, the \(V_{rev}\) is the prepulse potential, \(V_{1/2}\) is the potential at which inactivation is half-maximal, and \(k\) is the slope factor.

**Drug Application**

*In vivo* systematic administration of B1 (66 mg/kg, 0.1 ml, intraperitoneal) was made immediately after surgery and then once daily for a consecutive 10–14 days, including the period of electrophysiological recordings during postoperative days 10–14. *In vivo* topical administration of B1 (1, 5, and 10 ms) into the IVF at \(L_1\) and \(L_4\) was made *via* the silicon tubing connected to the hollow rod. In the *in vitro* studies, B1 was added to the buffered solution bathing the dissociated DRG neurons 50–60 min before recording. B1 was applied at 0.1, 1, and 10 μM, respectively. Saline and phosphate buffer solution were injected, respectively, as controls.

**Statistical Analysis.** SPSS Rel 15 (SPSS Inc., Chicago, IL) and CLAMPFIT V10.0 (Molecular Devices) were used to conduct all the statistical analyses and the model fitting procedures, respectively. Differences in mean latency of thermal paw withdrawal over time were tested with repeated-measures analysis of variance (RM ANOVA). After demonstrating that the data were normally distributed using the Shapiro-Wilks’ W test, a two-way RM ANOVA followed by Bonferroni post hoc tests involving the treatment factors and postoperative days were used to test the significance of difference in withdrawal latency between experimental conditions. One-way ANOVA followed by Bonferroni post hoc tests was used to test the hypothesis that RMP, excitability (including the AP current threshold and number of APs evoked by 1 s pulse), and the sodium current properties of DRG neurons in CCD groups were different from those CCD groups with treatment of vitamin B1 at different doses. Chi-square tests were used to identify differences in the incidence of effects. All data are presented as means and standard errors of the mean (mean ± SE). Unless otherwise stated, results described as significant are based on a criterion of \(P < 0.05\).

**Results**

*B1 Inhibits CCD-induced Thermal Hyperalgesia*

We began by confirming and extending our earlier demonstrations that B1 inhibits thermal hyperalgesia produced by CCD treatment.\(^{13}\) All CCD-treated rats used later for

---

Anesthesiology, V 110, No 2, Feb 2009

---

Copyright © by the American Society of Anesthesiologists. Unauthorized reproduction of this article is prohibited.
electrophysiological analysis showed clear behavioral indications of thermal hyperalgesia (fig. 1). As shown in figure 1A, withdrawal latencies of the feet ipsilateral to CCD treatment were significantly decreased from the preoperative values (mean of the two preoperative tests) each day after surgery, through the last test on the day of electrophysiological recording (10-14 days after surgery) \( (P < 0.01, t \text{ test}, n = 18) \). Withdrawal latencies of the feet ipsilateral to sham-operation \( (n = 18) \) were not significantly changed before and after surgery. The contralateral feet in CCD- and sham-operated rats did not show significant change during the same period, except for a slightly shortened latency in the CCD rats on day 1 and 3 \( (P > 0.05) \). Repetitive systematic administration of B1 (intraperitoneal, 66 mg/kg, first dose given immediately after surgery, then once daily for consecutive 10-14 days until the day of electrophysiological recording) significantly reduced CCD-induced thermal hyperalgesia, whereas the same treatments did not change the thermal sensitivity in the sham-operated animals (fig. 1B). Withdrawal latencies of the contralateral feet were not significantly altered by the CCD-, sham-operation-, B1- and/or saline-treatment in these animals (data not shown). These results indicate that repetitive application of B1 alone can produce inhibitory effects on CCD-induced thermal hyperalgesia that are similar to the effects produced by a combination of B1, B6, and B12.13 The rats tested behaviorally were then used for electrophysiological studies 10-14 days after surgery and/or the treatments described.

In order to further test the pharmacological effects of vitamin B1 administrated \textit{in vivo}, we investigated the effects of B1 on thermal hyperalgesia by topically applied B1 at 1, 5, and 10 mM into the IVF at L4 and L5. Our data showed that administration of the B1 again produced significant inhibitory effects on the thermal hyperalgesia in a dose-related manner, \textit{i.e.}, B1 at 1 mM produced transient inhibition (20–50%) of thermal hyperalgesia during 2–6 h after injection, while B1 at 5 and 10 mM suppressed the hyperalgesia by 30–65% during 2–24 h after injection. Data are summarized in figure 1C. There was no obvious toxicity observed in the rats that received the IVF application of B1.

**B1 Inhibits CCD-induced Hyperexcitability in All Sizes of DRG Neurons**

Nerve injury-induced DRG neuron hyperexcitability contributes to the sensitization of central nociceptive neurons in the spinal dorsal horn, leading to chronic pain and hyperalgesia. Hyperexcitability of DRG neurons after CCD treatment and other forms of injury is often manifested as RMP depolarization, a decrease in AP current threshold, increased repetitive discharge, and spontaneous activity.19–23,35–37 We examined these four electrophysiological properties to test the possibility that B1 may reduce thermal hyperalgesia by

Anesthesiology, V 110, No 2, Feb 2009
inhibiting DRG neuron hyperexcitability after CCD treatment.

Intracellular recordings were made from 185 large, 371 medium, and 211 small neurons in intact DRGs from CCD and sham-operated rats. The CCD DRG neurons, compared to those from sham operated ganglia, were more excitable, as manifested by significant RMP depolarization, decreased AP threshold current and increased rate of repetitive discharges during depolarization, and in the incidence of spontaneous activity. In vitro bath application and in vivo repetitive injection (intraperitoneal) of B1 significantly suppressed the hyperexcitability of the injured DRG neurons. Examples are given in figure 2. Further, as shown in figure 3, in vitro administration of B1 at 0.1 mM, 1 mM, and 10 mM, respectively, produced significant inhibitory effects on the hyperexcitability of CCD DRG neurons in a dose-dependent manner. B1 at 10 mM significantly restored the depolarized RMP, reversed the decreased AP current threshold, increased repetitive discharges, and increased the incidence of spontaneous activity in all three categories of neurons; B1 at 1 mM significantly inhibited the neural hyperexcitability in the small and medium-sized but not large DRG neurons; while B1 at 0.1 mM did not affect excitability of the neurons in any category. Only the highest dose of B1 (10 mM) suppressed the enhanced excitability of the large neurons and significantly restored the depolarized RMP in all the three categories of DRG neurons. B1 at 1 and 10 mM did not significantly affect the RMP and excitability of different sizes of DRG neurons (large: 26; medium: 34; small: 24) from sham-operated rats (data not shown).

Repetitive injection of B1 (66 mg/kg, intraperitoneal, given immediately after surgery and then once daily consecutively for 10–14 days until the day of electrophysiological recording), which successfully reduced severity and shortened duration of thermal hyperalgesia (see fig. 1B), also significantly inhibited hyperexcitability of the small-, medium-, and large-sized neurons (fig. 3). These results indicate that B1 can effectively reduce the CCD-induced DRG neuron hyperexcitability that probably contributes to hyperalgesia by causing central sensitization.

Recordings of the TTX-R and TTX-S Na+ Currents in Small DRG Neurons

Given that B1 can suppress behavioral thermal hyperalgesia and DRG neuron hyperexcitability, an interesting
Fig. 3. In vitro and in vivo applications of vitamin B1 suppress hyperexcitability of the dorsal root ganglion (DRG) neuron after chronic compression of DRG (CCD). A: Effects on resting membrane potentials (RMP). B: Effects on the current needed to reach action potential (AP) threshold. C: Effects on repetitive firing evoked by a 1-s depolarizing pulse at 2.5 × the threshold current. D: Percentage of tested DRG neurons displaying spontaneous activity (SA). *P < 0.05, **P < 0.01 indicate significant differences compared to the sham control group. #P < 0.05, ##P < 0.01 indicate significant differences between corresponding groups in the presence and absence of vitamin B1. The numbers of cells tested in each group are shown in parentheses. B1 was applied in vitro at 0.1, 1, and 10 mM, respectively, and in vivo repetitive intraperitoneal injection (66 mg/kg, once daily consecutive for 10–14 days).
question is whether B1 can regulate the TTX-R and/or TTX-S Na\(^+\) currents in the chronically compressed DRG neurons, because the VGSCs have been considered to play an important role in modulating DRG neuron hyperexcitability. The data presented in the preceding section showed that the small DRG neurons are most sensitive to B1. Because TTX-R and TTX-S Na\(^+\) currents are expressed in most of the small DRG neurons,\(^{38}\) we investigated possible changes of the TTX-R and TTX-S Na\(^+\) currents in small DRG neurons after CCD and CCD plus B1 treatment by means of whole-cell patch-clamp recordings. Since\(^{in vitro}\) application of B1 at 1 mM produced significant effects on hyperexcitability of injured small DRG neurons, we used this concentration to test B1’s effects on Na\(^+\) currents in small DRG neurons.

Prepulse inactivation, which takes advantage of differences in the inactivation properties of the TTX-R and TTX-S currents, was used to separate the slow-inactivating TTX-R and fast-inactivating TTX-S Na\(^+\) currents.\(^{24,38,39}\) A current-voltage protocol with a 700 ms prepulse to \(-120\) mV followed by respective test pulse was applied first at a holding potential of \(-80\) mV to obtain the total Na\(^+\) current. The slow-inactivating TTX-R currents were recorded using a prepulse of 700 ms \(-50\) mV before the test pulse. This protocol inactivates the TTX-S currents while leaving the TTX-R current intact. TTX-S currents were then obtained by subtracting the TTX-R currents from the total Na\(^+\) current in the cells. These protocols allowed simultaneous measurement of both TTX-R and TTX-S currents in each neuron recorded. Examples are shown in figure 4A–C. Current-voltage relationships of the TTX-R and TTX-S Na\(^+\) currents were measured using an I-V protocol with 700 ms prepulse to \(-120\) or \(-50\) mV, followed by a series of test pulses from \(-70\) to \(+60\) mV with \(+10\) mV increments. The total Na\(^+\) current was obtained by using the protocol with a prepulse to \(-120\) mV. The TTX-R component was recorded by using the protocol with a prepulse to \(-50\) mV, which inactivates TTX-S currents. The TTX-S component (fig. 4C) was obtained by digitally subtracting the TTX-R component (fig. 4B) from the total Na\(^+\) current (fig. 4A). Steady-state inactivation of TTX-R and TTX-S Na\(^+\) currents was measured with 500 ms prepulse to potentials over the range of \(-120\) mV to \(-10\) mV with 5 mV increments followed by a \(-10\) mV test pulse with a 0.8 ms interpulse interval to \(-80\) mV. The TTX-R inactivated currents were measured at the time of the peak current evoked following a \(-50\) mV prepulse. The TTX-S inactivated currents were obtained by subtracting the currents obtained at a \(-50\) mV prepulse from the current obtained at the more hyperpolarized prepulse potential and measured at the time of the peak of the maximum current evoked following a \(-120\) mV prepulse (for method, see ref.\(^{39}\)). An example is given in figure 4D.
B1 Reverses CCD-induced Alterations of TTX-R Na⁺ Current in Small DRG Neurons

The densities (peak current amplitude normalized to $C_m$) and the voltage dependence of steady-state activation of TTX-R Na⁺ currents were examined and compared in the small DRG neurons among groups of sham, CCD, and CCD with in vitro perfusion or in vivo intraperitoneal injection of vitamin B1. *B-E: Effects of in vitro and in vivo application of B1 on the density (B), current-voltage (I-V) relationship (C), activation (D), and steady-state inactivation (E) of TTX-R Na⁺ currents of the small DRG neurons after CCD treatment. In B: * $P < 0.05$ indicates significant differences compared with the sham-operated control group. # $P < 0.05$ indicates significant differences compared with the CCD group. The current I-V relationship (C) and activation (D) were not altered by CCD and B1 treatment. The current inactivation curve (E) was left-shifted (hyperpolarized) by CCD and corrected by in vitro application of B1. The numbers of cells tested in each group are shown in parentheses.

**Fig. 5.** Effects of vitamin B1 on the tetrodotoxin-resistant (TTX-R) Na⁺ current densities and properties of the small dorsal root ganglion (DRG) neurons after chronic compression of DRG (CCD). A: Representative TTX-R Na⁺ current traces recorded in small neurons from sham, CCD, and CCD with in vitro perfusion or in vivo intraperitoneal injection of vitamin B1. B–E: Effects of in vitro and in vivo application of B1 on the density (B), current-voltage (I-V) relationship (C), activation (D), and steady-state inactivation (E) of TTX-R Na⁺ currents of the small DRG neurons after CCD treatment. In B: * $P < 0.05$ indicates significant differences compared with the sham-operated control group. # $P < 0.05$ indicates significant differences compared with the CCD group. The current I-V relationship (C) and activation (D) were not altered by CCD and B1 treatment. The current inactivation curve (E) was left-shifted (hyperpolarized) by CCD and corrected by in vitro application of B1. The numbers of cells tested in each group are shown in parentheses.

*Song ET AL.*

Anesthesiology, V 110, No 2, Feb 2009
Table 1. Effects of In Vitro and In Vivo Application of Vitamin B1 on the Voltage Dependence of Activation and Steady-state Inactivation of the TTX-R Na\(^+\) Currents of the Small Dorsal Root Ganglion Neurons in Rats with CCD Treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>V(_{1/2}) (mV)</th>
<th>k</th>
<th>n</th>
<th>V(_{1/2}) (mV)</th>
<th>k</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>18</td>
<td>-15.3 ± 1.4</td>
<td>4.90 ± 0.30</td>
<td>14</td>
<td>-36.1 ± 1.0</td>
<td>4.06 ± 0.11</td>
</tr>
<tr>
<td>CCD</td>
<td>15</td>
<td>-15.1 ± 1.8</td>
<td>5.37 ± 0.45</td>
<td>16</td>
<td>-39.8 ± 1.4*</td>
<td>-4.56 ± 0.18*</td>
</tr>
<tr>
<td>CCD + B(_1) (1 mm)</td>
<td>12</td>
<td>-16.1 ± 1.6</td>
<td>4.10 ± 0.22</td>
<td>11</td>
<td>-36.3 ± 1.6†</td>
<td>-3.89 ± 0.19†</td>
</tr>
<tr>
<td>CCD + B(_1) (i.p.)</td>
<td>13</td>
<td>-17.8 ± 1.9</td>
<td>4.53 ± 0.58</td>
<td>13</td>
<td>-37.9 ± 1.8</td>
<td>-3.59 ± 0.27†</td>
</tr>
</tbody>
</table>

\(P < 0.05\) versus group of sham; † \(P < 0.05\) versus group of chronic compression of dorsal root ganglion (CCD).

TTX-R = tetrodotoxin resistant.

Na\(^+\) current was not altered by either CCD or B1 treatment (fig. 5C and D; table 1). However, the steady-state inactivation of the TTX-R Na\(^+\) current was significantly altered by CCD treatment. The inactivation curve was left-shifted (hyperpolarized), and therefore the midpoint (V\(_{1/2}\)) and slope (k) of the curve were reduced. Such reduction of V\(_{1/2}\) and k was reversed by in vitro application of B1 (1 mm) (fig. 5E; table 1). In vivo repetitive administration of B1 reversed the reduced k and tended to correct the reduced V\(_{1/2}\) (table 1), although the latter effect was not statistically significant.

**B1 Fails to Affect CCD-induced Alterations of TTX-S Na\(^+\) Current in Small DRG Neurons**

The densities (peak current amplitude normalized to Cm) and the voltage dependence of steady-state activation of TTX-S Na\(^+\) currents were also examined and compared in the small DRG neurons among groups of sham, CCD, and CCD with or without B1 treatment (fig. 6). The peak TTX-S current amplitudes were measured with a 40 ms test pulse to -10 mV. The results showed that the TTX-S current densities were significantly reduced after CCD treatment by approximately 50% (fig. 6A, B). In contrast to the effect on the density of the TTX-R Na\(^+\) current, down-regulation of the TTX-S Na\(^+\) current densities were not significantly affected by B1 treatment (\(P > 0.05\), fig. 6A, B). The voltage dependence of steady-state activation of the TTX-S Na\(^+\) current was affected by neither CCD nor B1 treatment (fig. 6C and D, table 2). The steady-state inactivation of TTX-S Na\(^+\) current was altered by CCD treatment as evidenced by the reduced V\(_{1/2}\). Both in vitro and repetitive in vitro treatment of B1 failed to alter the reduced V\(_{1/2}\) (fig. 6E, table 2).

**B1 Inhibits Increase of Ramp Currents in the Small CCD DRG Neurons**

The inward ramp current produced by slow depolarization below AP threshold represents a depolarizing response to stimuli that have not reached AP threshold. Under our conditions, this ramp current is likely to be carried largely and possibly entirely by Na\(^+\). Cells with increased ramp currents have an augmented response to subthreshold depolarizing input, which could lead to a reduced firing threshold.\(^{40,41}\) Therefore, the ramp current might also influence the DRG neuron excitability after CCD treatment. We further examined effects of CCD on the ramp currents in the small DRG neurons and effects of B1 on CCD-induced alteration of the ramp currents. As shown in figure 7A, the ramp currents were elicited in response to a protocol (inset) with a 700 ms, -120 mV prepulse followed by a depolarizing voltage ramp (=0.23 mV/ms) from -120 mV to +40 mV over 695 ms. CCD treatment resulted in a significant increase in the ramp currents. Further, such CCD-induced increase of the ramp currents was significantly reduced (55–60%) by either in vitro repetitive administration (66 mg/kg, intraperitoneal, once daily for 10–14 days) or in vitro treatment (1 ms) of vitamin B1. Examples are given in Fig. 7A and B and data summarized in figure 7C. The ramp currents were normalized to peak transient currents elicited by -10 mV pulse calculated as \([- \times (\text{Ramp Current/Total Na}^+ \text{ Current}) \times 100\%]\) (B and C). These findings show that B1 can partially reverse alterations of Na\(^+\) currents in injured DRG neurons.

**Discussion**

The present study investigated effects of vitamin B1 on neural excitability and Na\(^+\) currents in DRG neurons altered by CCD treatment. The principle findings are 1) repetitive in vitro intraperitoneal administration of B1 significantly inhibits CCD-induced DRG neuron hyperexcitability, in addition to suppressing thermal hyperalgesia; 2) in vitro treatment of B1 inhibits CCD-induced neural hyperexcitability in a dose-dependent manner; 3) the small and medium-sized neurons exhibit higher sensitivity than the large-sized neurons to B1 treatment; 4) B1 treatment can partially reverse injury-induced alterations of densities and inactivation properties of TTX-R and TTX-S Na\(^+\) currents and ramp currents in small DRG neurons. These findings demonstrate, for the first time, that vitamin B1 can modulate neural excitability and Na\(^+\) currents in injured DRG neurons, in addition to suppressing thermal hyperalgesia, and begin to address mechanisms in these neurons that may contribute to the analgesic effects of B1 in neuropathic pain. This study supports the
promise of B vitamins for treating neuropathic painful conditions following injury, inflammation, degeneration, or other disorders in the human nervous system.

Although the concept that B vitamins can produce analgesic effects in several painful conditions has been supported by experimental and some clinical evidence for decades, there was no direct experimental evidence of the use of B vitamins to treat neuropathic pain until an article published in 2005. By using well-established models of neuropathic pain after sensory neuron injury, we first demonstrated that vitamins B1, B6, and B12 can significantly reduce the severity and shorten the duration of thermal hyperalgesia in rats with peripheral nerve injury or DRG compression. Recently, it was further shown that benfotiamine, a lipid-soluble analogue of B1, can relieve hyperalgesia in rats with diabetes. These findings demonstrate an analgesic effect of the B vitamins in neuropathic pain and encour-

Fig. 6. Effects of vitamin B1 on the tetrodotoxin-sensitive (TTX-S) Na⁺ current densities and properties of the small dorsal root ganglion (DRG) neurons after chronic compression of DRG (CCD). A: Representative TTX-S Na⁺ current traces recorded in small neurons from sham operated, CCD, and CCD with B1 treatment (in vitro or in vivo, intraperitoneal). B-E: Effects of in vitro and in vivo application of B1 on the current current-voltage (I-V) relationship (C), activation (D), and steady-state inactivation (E) of TTX-S Na⁺ currents of the small DRG neurons after CCD treatment. In B: * P < 0.05 indicates significant differences compared with the sham-operated control group. There were no significant difference among groups of CCD and CCD plus B1 treatments. CCD treatment caused right-shift of the inactivation (E), but not the I-V curve (C) and activation (D) of the TTX-S Na⁺ currents. In vitro treatment of B1 altered the current I-V curve, activation and inactivation as shown in C-E. The numbers of cells tested in each group are shown in parentheses.
The present study provides the first evidence that vitamin B1-induced inhibition of DRG neuron hyperexcitability may contribute to B1-induced suppression of thermal hyperalgesia in an animal model of neuropathic pain. It has been well documented that nerve injury and/or compression and inflammation can produce DRG neuron hyperexcitability, which is thought to underlie neuropathic pain by causing central sensitization. Therapeutic agents that suppress behavioral hy-

**Table 2. Effects of In Vitro and In Vivo Application of Vitamin B₁ on the Voltage Dependence of Activation and Steady-state Inactivation of the TTX-S Na⁺ Currents of the Small Dorsal Root Ganglion Neurons**

<table>
<thead>
<tr>
<th></th>
<th>Voltage Dependent Activation</th>
<th></th>
<th>Steady-state Inactivation</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>$V_{1/2}$ (mV)</td>
<td>$k$</td>
<td>n</td>
</tr>
<tr>
<td>Sham</td>
<td>16</td>
<td>-25.8 ± 1.1</td>
<td>5.06 ± 0.39</td>
<td>14</td>
</tr>
<tr>
<td>CCD</td>
<td>14</td>
<td>-25.0 ± 1.2</td>
<td>4.58 ± 0.42</td>
<td>16</td>
</tr>
<tr>
<td>CCD + B₁ (1 mM)</td>
<td>12</td>
<td>-24.8 ± 1.1</td>
<td>5.23 ± 1.88</td>
<td>11</td>
</tr>
<tr>
<td>CCD + B₁ (i.p.)</td>
<td>13</td>
<td>-26.5 ± 2.8</td>
<td>5.07 ± 0.91</td>
<td>13</td>
</tr>
</tbody>
</table>

$^* P < 0.05$ versus group of sham.

CCD = chronic compression of dorsal root ganglion; TTX-S = tetrodotoxin sensitive.

Fig. 7. Effects of vitamin B₁ on Na⁺ ramp currents of the small dorsal root ganglion (DRG) neurons after chronic compression of DRG (CCD). Representative total Na⁺ currents and Na⁺ ramp currents records elicited by a protocol (inset) with 700 ms −120 mV prepulse followed by 695 ms voltage ramp from −120 mV to +40 mV (−0.23 mV/ms) in sham control, CCD and CCD plus B₁ treatment in vitro or in vivo are shown in A. To compare changes of Na⁺ ramp currents among different groups, the ramp current was normalized to peak transient current of the total Na⁺ currents elicited by −10 mV pulse calculated as [(Ramp Current/Total Na⁺ Current) × 100%]. Examples are given in B and data summarize in C. $^* P < 0.05$ indicates significant differences compared with the sham-operated control group. $^# P < 0.05$ indicates significant differences compared with the CCD group.
eralgesia and/or allodynia can reduce the DRG neuron hyperexcitability; conversely, hyperexcitability of the affected DRG neurons significantly decreases while the hyperalgesia and/or allodynia are reduced.20,25,45,46 The results here demonstrate that vitamin B1 can significantly suppress DRG neuron hyperexcitability while reducing thermal hyperalgesia, suggesting that B1 may relieve the hyperalgesia by regulating neural excitability. Furthermore, the manner of dose-dependent inhibition of B1 on neural hyperexcitability may provide a cellular basis for the dose-dependent inhibitory effect of B vitamins on behavioral thermal hyperalgesia we have reported recently.15 In addition, our results show that large DRG neurons exhibit significantly less sensitivity to the B1 treatment than medium-sized and small neurons. This finding may provide an explanation for the behavioral observations that in vivo administration of B vitamins inhibits thermal hyperalgesia, but not mechanical allodynia in rats with chronic constriction injury of the sciatic nerve or DRG compression.13 Spontaneously active Aβ-fibers and hyperexcitability in large DRG neurons have been considered to contribute to the mechanical allodynia after nerve injury.18 It would be interesting to further investigate the possible mechanisms underlying the differential effects of B vitamins on thermal and mechanical hypersensitivity.

The VGSCs are essential for neural excitability and impulse conduction, and can be dynamically regulated by axonal injury, DRG compression, and peripheral inflammation.16,24–26 VGSCs are also a target of some analgesics. Studies have shown that interfering with expression of some of the VGSCs can effectively suppress neuropathic pain.25,29–31,47 Here we show that vitamin B1 can significantly reverse and/or modulate CCD-induced alterations of densities and/or inactivation properties of the TTX-R Na+ currents. These effects of B1 on Na+ currents, however, would not be expected to decrease hyperexcitability, and might even increase excitability of the DRG neurons. Therefore, the associated effects of B1 on Na+ currents have an uncertain relationship to the effects of B1 on neuropathic pain. On the other hand, the vitamin B1-induced reduction of the increased ramp currents (fig. 7), which are likely to be carried largely and possibly entirely by Na+, may contribute to B1-induced inhibition of the neural hyperexcitability. In contrast to what happens with the TTX-R Na+ currents, alterations of the density and inactivation properties of the TTX-S Na+ currents are not affected significantly by the B1 treatment, suggesting complex effects of B1 on the sodium channels after neuronal injury. This finding, in addition to the difference between the large and small/medium-sized DRG neurons in B1 sensitivity, may help explain the different effects of B vitamins on thermal and mechanical hypersensitivity. It is known that large DRG neurons predominantly express TTX-S Na+ currents, but B1 does not significantly affect the TTX-S currents in the DRG neurons after injury. The ability of B1 to reduce the CCD-induced DRG hyperexcitability is a potentially important finding, while the observed effects on Na+ currents following B1 application have undetermined roles in the associated effects on excitability and behavior. Further studies are needed to clarify the possible relationships between the Na+ currents/channels and the neural hyperexcitability, behavioral thermal hyperalgesia, and mechanical allodynia. The B vitamins exhibit antinociceptive effects in animals with inflammatory pain5 or neuropathic pain, but only at higher doses.15,14 Our recent study13 shows that a single treatment of B1 at 5, 10, 33, and 100 mg/kg can produce transient, significant analgesic effects on CCD- or nerve injury-induced thermal hyperalgesia. The highest dose of B1 given in vivo in the present study (66 mg/kg) is approximately eight times the recommended maximum daily dose for humans, 8.3 mg/kg (assuming an average body weight of 60 kg).48,49 Similar doses (5 and 10 mg/kg/day) have also been given in clinical studies on analgesia.50 Fortunately, B1 given alone or in combination with B6 and B12 up to an oral dose of 5000 mg/kg body weight49 in human adults has shown minimal or no toxicity in 5–10 yr of study, although 500–1000 mg/day shows peripheral neuropathy within 1–3 yr.51 In clinical treatment of certain chronic pain, high doses of B1 or B6 and 12 are sometimes mixed with a local anesthetic such as lidocaine and injected topically into the tissues, which are adjacent to the targeted nerves or the DRG within the IVF. Such an injection, which is usually expected to provide nutrition to the injured and/or inflamed nerve tissues, does not cause toxicity. In the present study, in vitro perfusion of DRG with B1 produced dose-dependent inhibition of DRG neuron hyperexcitability, i.e., B1 at 0.1 mM did not produce the effect, 1 mM produced a moderate effect, and 10 mM produced the greatest effect. Furthermore, in vivo topical application of high doses of B1 (1, 5, and 10 ms) into the IVF also produced dose-dependent inhibition of thermal hyperalgesia without observed toxicity in the rats. It is known that blood concentrations of B1 in healthy humans and rats are within similar ranges, 0.8–2.8 μg/L.52–55 We, therefore, have good reason to recommend doses of vitamin B1 that are much higher than the physiologic level or the currently recommended average or maximum daily doses for humans48,49 in order to achieve the best treatment effect. Higher non-physiological doses of vitamin B may be practical in the clinic and of interest for the treatment of neuropathic pain and probably other painful conditions, although current results from clinical trials are unclear.

In addition to the present findings that B1 can reverse hyperexcitability and alterations of sodium currents after neuronal injury, recent studies have implicated B1-dependent processes in oxidative stress, protein processing, peroxisomal function, and gene expression.56,57

Anesthesiology. V 110, No 2, Feb 2009

Copyright © by the American Society of Anesthesiologists. Unauthorized reproduction of this article is prohibited.
These processes are all important after nerve injury, which can alter properties of diverse types of neurons and trigger myriad changes in gene expression that affect many proteins, including ion channels, receptors, and other membrane proteins. These observations encourage further clinical investigation into the utility of the B vitamins in treating chronic pain and related disorders after nerve injury or disease.

References

49. Leuschner J: Antinociceptive properties of thiamine, pyridoxine and cyanocobalamin following repeated oral administration to mice. Arzneimittelforschung 1992; 42:114–5


