Impaired Nociception and Peripheral Opioid Antinociception in Mice Lacking Both Kinin B1 and B2 Receptors

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ABSTRACT

Background: Kinins (e.g., bradykinin) acting through the constitutively expressed B2 and the injury-induced B1 receptors are involved in pain and hyperalgesia, as previously shown by use of receptor-selective antagonists and single-receptor knockout models. Because the overall contribution of kinins to painful processes remains unclear, the aim of this study was to analyze pain-related behaviors of mice unable to respond to kinins because of a lack of both B1 and B2 receptors.

Methods: In knockout mice lacking both B1 and B2 receptors and in wild-type mice (n = 8–21 per group) the authors assessed nociceptive thresholds to mechanical and heat stimuli (von Frey and Hargreaves tests, respectively) in healthy animals and after induction of inflammatory and neuropathic pain, acid-induced visceral nociception, and modulation of nociceptive responses by peripherally administered opioid agonists.

Results: In knockout mice lacking both B1 and B2 receptors baseline nociceptive responses to heat were unaltered, nocifensive responses to bradykinin were abolished, acute acetic acid-induced visceral nociception was reduced by approximately 70% (mean difference: 19.5 writhes/30 min) and heat hypersensitivity in carrageenan-induced paw inflammation was decreased 48 h after injection (mean difference 2.88 s), hypersensitivities in chronic complete Freund's adjuvant-induced paw inflammation or after chronic constriction injury of the sciatic nerve were unchanged, and peripheral μ- and δ-opioid–induced analgesia after chronic constriction injury was reduced by 30–35% (mean differences: μ-agonist: 0.495 g, δ-agonist: 0.555 g).

Conclusions: These data suggest that kinins are important for nociception associated with acute short-lasting inflammation but are less essential in chronic stages of pain. The results also highlight a new protective function of kinins via interactions with the opioid system.

What We Already Know about This Topic

• Kinins, produced in response to tissue trauma and inflammation, are known to activate and sensitize peripheral sensory neurons and possibly to reduce responses to opioid analgesics
• Although nociceptive responses of mice devoid of individual bradykinin receptors (B1 or B2) have been examined, pain-related behaviors of mice devoid of both receptors have not been studied

What This Article Tells Us That Is New

• In mice lacking both B1 and B2 receptors, responses to acute somatic and visceral inflammation were reduced, but chronic inflammatory or nerve injury responses were unaltered
• Peripheral opioid analgesia was reduced in animals lacking both receptors

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evidence indicates that kinins are also involved in pain relief by augmenting peripheral opioid receptor function.7–9

Kinins act on two distinct G-protein coupled receptors, the constitutively expressed B2 and the injury-induced B1 receptors. The receptors differ in their pharmacologic properties, desensitization abilities, and expression profiles.10 The B2 receptor preferentially binds bradykinin and Lys-bradykinin and is constitutively expressed.10,11 The B1 receptor has a higher affinity for active kinin metabolites (des-Arg9-bradykinin, Lys-des-Arg9-bradykinin) and is weakly expressed under healthy conditions and strongly up-regulated in pathologic conditions.12–14

The role of each receptor in nociception has been studied extensively by using specific antagonists and genetically modified animals. Although B2 receptor antagonists induced antinociception in acute pain models15–17 and reduced inflammatory hypersensitivity,15,18–20 studies in mice lacking the B2 receptor (B2KO) showed only a modest contribution of the B2 receptor to nociception; baseline sensitivities, nociceptive responses to intraplantar injections of formalin or capsaicin,21,22 and hypersensitivity induced by complete Freund’s adjuvant (CFA)21,23,24 were unaltered in these mice. Nevertheless, hypersensitivity induced by carrageenan was attenuated.21,23

Because of its unique injury-induced pattern of expression, the B1 receptor is thought to play a role mostly in chronic phases of inflammation and pain.12 Specific blockade of B1 receptors was shown to reduce partially both carrageenan-20,21,25 and CFA-induced hypersensitivities.21,24,26,27 In addition, in mice lacking the B1 receptor (B1KO), nociceptive responses to formalin or capsaicin,28 CFA-induced hypersensitivity,24,29 and thermal hypersensitivity associated with nerve injury25,30–33 were reduced.

Although the role of each receptor in different nociceptive models has been examined broadly, these studies present discrepancies and limitations. Antagonists, especially peptidic compounds such as the classic B1 antagonist des-Arg9-[Leu6]-bradykinin, have high metabolic liability that precludes investigating long-term effects. Partial agonist activities have been described,34 and the specificity of the widely used B2 antagonist HOE140 was challenged.35 Mixed genetic backgrounds and compensatory effects mediated through the remaining receptor24,36 also limit studies in single receptor knockout animals. In addition, the overall contribution of kinins in painful processes remains unknown.

Therefore, the aim of the current study was to analyze pain-related behaviors of knockout mice unable to respond to kinins because of a lack of B1 and B2 receptors (B1/ B2KO).37 We hypothesized that thermal and mechanical sensitivities in untreated mice would not change, responses to acute visceral pain and hypersensitivity associated with inflammation or neuropathy would be decreased, and peripheral opioid analgesia in a model of neuropathic pain would be reduced.

Materials and Methods

Animals

Male knockout mice and wild-type (WT) age-matched controls were used in this study (25–30 g, 2–4 months old). B1/B2KO mice were generated by homologous recombination of the B1 receptor gene in B2-deficient embryonic stem cells.37 B1/B2KO mice were backcrossed eight times to C57BL/6N background, and C57BL/6N mice were used as WT controls. Experiments also were conducted in the single B1KO and B2KO mice backcrossed to C57BL/6J. Mice were bred at Charité-Universitätsmedizin Berlin, Campus Benjamin Franklin (Berlin, Germany); they were housed under controlled temperature, humidity, and lighting (light–dark 12:12 hr cycle) with food and water available ad libitum. The genotypes of all mice were confirmed by polymerase chain reaction using primers amplifying a 170 pb fragment of the hygromycin resistance gene for B1/B2KO, primers amplifying a 280 pb fragment of the neomycin resistance gene for both B1KO and B2KO mice, and primers amplifying a 1,100 pb fragment overlapping the B1 gene and the neomycin resistance gene for B1KO mice. Experiments were approved by the local animal care committee (Berlin, Germany) and performed in accordance with the guidelines of the International Association for the Study of Pain. In all experiments, the investigator was blinded to the genotype.

Assessment of Mechanical and Thermal Sensitivities

Animals were habituated to the test cages daily starting 6 days before testing. Behavioral responses were recorded from both ipsilateral and contralateral paws before and after injection of nociceptive agents or chronic constriction injury (CCI) of the sciatic nerve at indicated time points (n = 8 per group). Two days after CCI, antinociceptive effects of opioid-receptor agonists were evaluated 30 min after near nerve injections (n = 8 per group).

Von Frey Test. Tactile sensitivities were evaluated with calibrated von Frey filaments (Stoelting Co., Wood Dale, IL) applied to the planter surface of the hind paws. The 50% mechanical threshold (in grams) was determined using the up-down method.39 Testing began using a 3.9 mN hair (0.4 g). If the animal withdrew the paw, the weaker hair was applied. In the case of no withdrawal, the next-stronger hair was applied. The maximum number of applications was six to nine, and the cutoff was 39.2 mN (4 g), as previously described.39

Hargreaves Test. Heat sensitivities were evaluated using the Hargreaves test.40 The latency (in seconds) required to elicit paw withdrawal was measured with an electronic timer (IITC Inc. Life Science, Woodland Hills, CA) after the application of radiant heat to the plantar surface of a hind paw from underneath the glass floor with a high intensity light bulb. The stimulus intensity was adjusted to yield baseline paw withdrawal latency of 10–12 s in noninflamed paws, and the cutoff was 20 s to avoid tissue
damage. The average of two measurements taken with a 1-min interval was calculated.

**Acetone Test.** To assess the sensitivity to cool temperatures, a drop of acetone (40 μl) was placed on the dorsal surface of the hind paw and behaviors were monitored. As acetone evaporates, it produces a cooling sensation. The response was ranked as 0 = no response; 0.5 = paw lifting; 1 = paw lifting; 1.5 = lifting and licking; 2 = flinching; 3 = flinching and licking; and the average was calculated.

**Bradykinin Injection**
Mice were habituated to observation boxes, briefly anesthetized with isoflurane (Abbott, Wiesbaden-Delkenheim, Germany), and bradykinin was injected intraplantarly (30 nmol/paw) in a volume of 20 μl. Nociceptive reactions (number of flinches) were measured for 25 min.

**Acetic Acid-induced Writhing Assay of Visceral Pain**
Mice were habituated to observation boxes and received injections of 0.6% (v/v) acetic acid intraperitoneally (300 μl/30 g body weight). The number of abdominal constrictions (“writhes”) was counted during a 30-min observation period.

**Carrageenan- and CFA-induced Paw Inflammation**
Animals were briefly anesthetized with isoflurane, and paw inflammation was induced by intraplantar injection of α-carrageenan (2% w/v, 20 μl) or CFA (50 μg heat-killed *Mycobacterium butyricum* in 20 μl Freund’s incomplete adjuvant) into the right hind paw. Paw volumes were measured by use of a plethysmometer (Ugo Basile, Comerio, Italy) by averaging two consecutive trials, before and every 24 h after CFA injections.

**Peripheral Nerve Injury**
Peripheral nerve damage was induced by CCI of the sciatic nerve. In anesthetized mice (isoflurane), the sciatic nerve was exposed at the level of the right midthigh, and three loose 4/0 silk ligatures were placed with approximately 1-mm spacing around the nerve. They were tied until they elicited a brief twitch in the respective hind limb. The wound was closed with silk sutures. For sham controls, the sciatic nerve was exposed but not ligated.

**Peripheral Injections of Opioid Agonists**
Two days after CCI, the μ-receptor agonist [D-Ala2,N-Me-Phe4,Gly5-ol]-enkephalin (DAMGO; 2 μg/mouse), the δ-agonist D-Pen2,D-Pen5-enkephalin (DPDPE; 150 μg/mouse), and the κ-agonist trans-(-)-3,4-dichloro-N-methyl-N-2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide (U50488; 50 μg/mouse) were applied in a volume of 30 μl near the nerve at the site of nerve injury under brief isoflurane anesthesia, as described previously. The von Frey test was performed before CCI and 2 days later, before and 30 min after agonist injections. The doses of agonists and the time of treatment were found to be most effective and restricted to the periphery in our previous experiments (Labuz D., Ph.D., unpublished data, February 2009, Berlin, Germany, dose response and kinetics of the effect for the three opioid agonists were provided).

**Statistical Analysis**
Results are expressed as mean ± SEM. Two-sample comparisons were made using the two-tailed Student unpaired t tests for independent normally distributed data. Multiple comparisons were evaluated with one-way ANOVA for normally distributed data. Two-way repeated measures ANOVA was used for multiple repeated measures to compare two treatments. Post hoc comparisons were performed using Bonferroni test. No data were missing for any of the variables. Differences were considered statistically significant if *P* < 0.05 (PRISM; GraphPad Software, Inc., La Jolla, CA).

**Drugs**
Bradykinin was obtained from Bachem (Weil am Rhein, Germany), heat-killed *M. butyricum* and Freund’s incomplete adjuvant from DIFCO/Becton Dickinson (Heidelberg, Germany). Opioid agonists (DAMGO, DPDPE, U50488), α-carrageenan, and acetic acid were purchased from Fluka/Sigma-Aldrich Chemie (Taufkirchen, Germany).

**Results**

**Baseline Sensory Responses Remain Unaltered in B1/B2KO Mice**
In untreated animals, paw withdrawal latencies to heat and von Frey thresholds were comparable between B1/B2KO and WT mice (*P* > 0.05, fig. 1A, B). Similarly, B1KO and B2KO mice showed no statistically significant differences compared with WT mice (*P* > 0.05; data not shown). These results show that a lack of kinin receptors does not affect baseline thermal and tactile sensitivities.

**Bradykinin Nocifensive Responses Are Abolished in B1/B2KO Mice**
In WT mice, intraplantar injection of bradykinin elicits short-lasting behavioral responses consisting of paw flinching and licking. B1/B2KO mice were insensitive to intraplantar bradykinin because flinches were almost absent in comparison with WT (*P* < 0.05, fig. 1C).

**B1/B2KO Mice Are Resistant to Acetic Acid-induced Visceral Pain**
In WT mice, intraperitoneal injection of acetic acid (0.6%) induced writhes (*i.e.*, characteristic lengthwise stretches of the body). The number of writhes counted in B1/B2KO mice was statistically lower than the number counted in WT mice (*P* < 0.01, fig. 1D). By contrast, no statistically significant differences were observed in B1KO mice. Although B2KO mice had fewer writhes, the difference did not reach significance.
was not statistically significantly different compared with that in WT mice (P > 0.05; two-way ANOVA repeated measures, fig. 2A). Hypersensitivities were also measured in the single knockout mice (table 1). Thermal hypersensitivity was statistically significantly diminished in B2KO mice at 6 and 48 h after carrageenan injection (P < 0.05) compared with that in the WT mice. Mechanical hypersensitivities in the B1KO and B2KO lines were not statistically significantly different compared with those in the WT (P > 0.05). Together, these results indicate that kinin receptors are partly involved in the development of thermal hypersensitivity induced by a mild inflammation.

**B1/B2KO Mice Develop Unaltered Hypersensitivities after CFA**

Intraplantar injection of CFA induces a stronger and longer lasting paw inflammation in comparison with that induced by carrageenan. In WT mice, paw withdrawal latencies in response to heat were statistically significantly decreased in the CFA-injected paw from 2 h until 5 days (P < 0.001) and returned to baseline values 7 days after injection (Hargreaves test, fig. 2B). In WT mice, mechanical hypersensitivity also was present from 2 h until 7 days after injection. In B1/B2KO mice, the development of thermal and mechanical hypersensitivities was similar to that in WT mice (P > 0.05, fig. 2B). In addition, paw volume increased in a comparable way between B1/B2KO and WT (P > 0.05, fig. 2B). Because some studies showed that B1 receptors were implicated in CFA-induced hypersensitivities, we tested the B1KO line but found no difference compared with the WT line (data not shown). These results indicate that kinin receptors alone are not critical for the development of thermal and mechanical hypersensitivities induced by CFA.

**B1/B2KO Mice Show Deficits in Carrageenan-induced Hypersensitivities**

In WT mice, paw withdrawal latencies to heat were statistically significantly reduced in the carrageenan-injected (ipsilateral) paw from 6 h until 48 h (P < 0.001) and returned to baseline values 72 h after injection (Hargreaves test, fig. 2A). In B1/B2KO mice, the overall response to carrageenan injection was statistically significantly reduced compared with WT (P < 0.05; two-way ANOVA repeated measures). Analyses of differences at each time point by Bonferroni test indicate that 48 h after injection, B1/B2KO paw withdrawal latencies were statistically significantly reduced compared with WT (P < 0.05). This reduction was associated with a faster recovery because ipsilateral latencies in B1/B2KO mice were back to baseline values 48 h after injection versus 72 h in WT mice. Carrageenan injection also reduced von Frey thresholds in WT mice for as long as 72 h (P < 0.05). In B1/B2KO mice, mechanical hypersensitivity

### Notes:

- **Acute nociceptive responses in B1/B2KO mice.**
- **Basal mechanical sensitivities von Frey test**
  - WT
  - B1/B2KO
- **Basal thermal sensitivities Hargreaves test**
  - WT
  - B1/B2KO
- **Intraplantar Bradykinin**
  - WT
  - B1/B2KO
- **Acetic acid-induced writhing**
  - WT
  - B1/B2KO

**Fig. 1.** Acute nociceptive responses in B1/B2KO mice. (A) Mechanical thresholds measured by von Frey test in B1/B2 knockout (B1/B2KO) mice and wild type (WT) mice (n = 21 per group). (B) Thermal thresholds measured with Hargreaves test in B1/B2KO and WT mice (n = 16 per group). (C) Bradykinin-induced nociception in B1/B2KO and WT mice (n = 8 per group). The number of flinches was measured for 25 min after intraplantar injection of BK (30 nmol/paw). (D) Acetic acid–induced writhing in B1/B2KO and WT mice (n = 8 per group). The number of writhes was measured for 30 min after intraperitoneal injection of acetic acid (0.6%). Student’s unpaired t tests (A, B, D) and two-way repeated measures ANOVA followed by Bonferroni test (C) were performed. *P < 0.05, **P < 0.01, ***P < 0.005 vs. WT. Blue bars: WT mice; red bars: B1/B2KO mice.

**B1/B2KO Mice Show Deficits in Carrageenan-induced Hypersensitivities**

In WT mice, paw withdrawal latencies in response to heat were statistically significantly reduced in the carrageenan-injected (ipsilateral) paw from 6 h until 48 h (P < 0.001) and returned to baseline values 72 h after injection (Hargreaves test, fig. 2A). In B1/B2KO mice, the overall response to carrageenan injection was statistically significantly reduced compared with WT (P < 0.05; two-way ANOVA repeated measures). Analyses of differences at each time point by Bonferroni test indicate that 48 h after injection, B1/B2KO paw withdrawal latencies were statistically significantly reduced compared with WT (P < 0.05). This reduction was associated with a faster recovery because ipsilateral latencies in B1/B2KO mice were back to baseline values 48 h after injection versus 72 h in WT mice. Carrageenan injection also reduced von Frey thresholds in WT mice for as long as 72 h (P < 0.05). In B1/B2KO mice, mechanical hypersensitivity

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**Statistical significance**

- 39 ± 4 writhes in B1KO, 17 ± 3 writhes in B2KO vs. 30 ± 5 in WT, P > 0.05, one-way ANOVA followed by Bonferroni post hoc test.

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**B1/B2KO Mice Develop Unaltered Hypersensitivities after Nerve Injury**

To gain insight into the role of kinins in neuropathy-related behaviors, sciatic nerve injury was induced by CCI in both WT and B1/B2KO mice. Thermal (heat and cool) and mechanical sensitivities were measured 2, 7, and 14 days after CCI. Similar to WT, B1/B2KO showed a strong reduction of thermal and mechanical thresholds from 2 days until 14 days after the injury (P < 0.001, fig. 3A). The hypersensitivities were not observed in sham-operated mice (data not shown). No statistically significant difference was measured between WT and B1/B2KO (P > 0.05), suggesting that kinin receptors alone are not critical for the development of hypersensitivity in this model of nerve injury.

**B1/B2KO Mice Show a Reduction of Peripheral μ- and δ-Opioid Induced Antinoception**

At 2 days after CCI, effects on nociceptive thresholds of peripherally applied selective agonists for μ-, δ-, and κ-opioid receptors were measured in B1/B2KO versus WT animals. At 30 min after agonist application at the site of nerve damage, paw with-
drawal thresholds significantly increased and returned to baseline thresholds (before CCI) in WT animals (P > 0.05 between baseline before CCI and 30 min after DAMGO, DPDPE, and U50488; fig. 3B). In B1/B2KO, DAMGO- and DPDPE-induced antinociceptive effects were statistically significantly reduced in comparison with WT (P < 0.05, fig. 3B). In contrast, the selective δ-agonist U50488 in B1/B2KO produced analgesic effects similar to those in WT (P > 0.05, fig. 3B). These results indicate that kinin receptors facilitate peripheral μ- and δ-opioid antinociceptive effects after nerve injury.

Discussion

To expand the knowledge of the overall role of kinin receptors in pain, mice lacking both kinin receptors were analyzed for the first time in terms of pain behaviors. Our results show

Table 1. Thermal and Mechanical Hypersensitivities after Carrageenan-induced Inflammation in B1KO, B2KO, and B1/B2KO Mice

<table>
<thead>
<tr>
<th>Time after Carrageenan Injection</th>
<th>0 h</th>
<th>6 h</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hargreaves test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>9.7 ± 0.6</td>
<td>4.0 ± 0.4</td>
<td>5.2 ± 0.7</td>
<td>6.4 ± 0.5</td>
<td>8.6 ± 0.4</td>
</tr>
<tr>
<td>B1/B2KO</td>
<td>10.3 ± 0.4</td>
<td>6.0 ± 0.8</td>
<td>6.6 ± 1.0</td>
<td>9.3 ± 0.8*</td>
<td>10.4 ± 0.7</td>
</tr>
<tr>
<td>B1KO</td>
<td>10.0 ± 0.5</td>
<td>4.1 ± 0.6</td>
<td>6.4 ± 0.6</td>
<td>7.4 ± 0.4</td>
<td>9.8 ± 0.7</td>
</tr>
<tr>
<td>B2KO</td>
<td>10.2 ± 0.3</td>
<td>6.1 ± 0.6*</td>
<td>5.5 ± 0.5</td>
<td>8.6 ± 0.6*</td>
<td>9.2 ± 0.4</td>
</tr>
<tr>
<td>Von Frey test</td>
<td></td>
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<tr>
<td>WT</td>
<td>1.75 ± 0.34</td>
<td>0.64 ± 0.08</td>
<td>0.85 ± 0.11</td>
<td>0.65 ± 0.20</td>
<td>0.68 ± 0.12</td>
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<tr>
<td>B1/B2KO</td>
<td>2.25 ± 0.15</td>
<td>1.02 ± 0.22</td>
<td>0.97 ± 0.21</td>
<td>1.24 ± 0.22</td>
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<tr>
<td>B1KO</td>
<td>2.37 ± 0.33</td>
<td>0.84 ± 0.16</td>
<td>1.01 ± 0.23</td>
<td>1.33 ± 0.32</td>
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<tr>
<td>B2KO</td>
<td>2.24 ± 0.19</td>
<td>0.94 ± 0.16</td>
<td>0.93 ± 0.17</td>
<td>0.90 ± 0.16</td>
<td>1.16 ± 0.19</td>
</tr>
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</table>

Thermal (Hargreaves test) and mechanical (von Frey test) thresholds were measured after intraplantar injection of carrageenan (2%, 20 μl/paw) in B1KO, B2KO, B1/B2KO, and WT mice. Data for B1/B2KO and WT mice were also used to generate figure 3. Two-way repeated measures ANOVA followed by Bonferroni test were performed.

* P < 0.05 vs. WT.

B1KO = knockout mice lacking the B1 receptor; B2KO = knockout mice lacking the B2 receptor; B1/B2KO = knockout mice lacking the B1 and B2 receptors; WT = wild-type mice.
that B1/B2KO mice are protected against acute acid-induced nociception and that short-lasting, carrageenan-induced heat hypersensitivity is diminished partially. Chronic hypersensitivity associated with CFA-induced inflammation and with injury of the sciatic nerve is not altered in B1/B2KO mice. In addition, peripheral analgesic effects of opioid agonists are reduced in B1/B2KO mice.

Baseline thermal and mechanical thresholds were unchanged in B1/B2KO mice, suggesting that in the absence of inflammation, B1 and B2 kinin receptors are not involved in the activation of thermal or mechanical nociceptors. This is in accordance with results in single knockout mice. It is worth noting that a temperature-dependent participation of the B1 receptor in the hot plate assay was suggested; however, this assay measures supraspinally integrated responses that are more complex than Hargreaves test responses.

The visceromotor response to intraperitoneal acetic acid is a well-established model for acute visceral pain. After intraperitoneal acetic acid, kinins are released in the peritoneum, where they can trigger the release of other algesic mediators such as prostaglandins or directly activate nociceptive neurons. The B2 receptors are localized in small and medium size sensory neurons. Bradykinin directly activates peripheral visceral afferent fibers, whereas B1 agonists do not. Even if basal B1 expression in sensory neurons is a matter of debate, the lack of effect in those studies probably is attributable to the very weak basal expression of the B1 receptor. Because writhing responses were diminished in the B2KO but not B1KO mice, the B2 receptor seems essential in relaying kinin effects. This is consistent with previous studies using B2 antagonists that showed involvement of capsaicin-sensitive C-fibers. Bradykinin itself induces writhing via the B2 receptor. The involvement of the B1 receptor cannot be completely excluded considering that the absence of B2 receptors alone did not affect the response as strongly as did the lack of both receptors. Porreca et al. also showed a reduced response to acetic acid in B1KO or in mice treated with the B1 antagonist LF 22–0542. The reasons for discrepancies between our results and those in B1KO mice could be attributed to different acetic acid doses (100 μl per mouse in the study by Porreca et al. vs. 200 μg acetic acid per 30 g mouse in our study).

Carrageenan injected intraplantarly induces paw inflammation and increases sensitivity to touch and heat. Because of its polysaccharide nature, carrageenan activates the contact

Fig. 3. Nerve injury–induced hypersensitivities and peripheral opioid antinociception in B1/B2KO mice. (A) Chronic constriction injury (CCI) of the sciatic nerve was induced and thermal thresholds were measured with Hargreaves test, mechanical thresholds by von Frey test, and sensitivities to cool temperatures by the acetone test. (B) Peripheral antinociceptive effects of opioid agonists were measured 2 days after CCI. Von Frey tests were performed before CCI and 2 days later, before and 30 min after near nerve injection of DAMGO (μ-receptor agonist, 2 μg/30 μl/mouse), DPDPE (δ-receptor agonist, 150 μg/30 μl/mouse), and U50488 (κ-receptor agonist, 50 μg/30 μl/mouse). Two-way repeated measures ANOVA followed by Bonferroni test were performed. * P < 0.05 vs. WT. N = 8 mice per group. Blue symbols: wild type (WT) mice; red symbols: B1/B2 knockout (B1/B2KO).
system of coagulation and leads to an increased tissue content of des-Arg⁹-bradykinin and bradykinin.⁵³,⁵⁴ both endogenous agonists of B1 and B2 receptors. In B1/B2KO mice, heat hypersensitivity was statistically significantly reduced compared with that in WT mice (Hargreaves test, fig. 2A). This difference, strongest at 48 h after carrageenan, was associated with a faster recovery in B1/B2KO mice than in controls. These results are in line with previous reports using selective blockade of B1 or B2 receptors in rats¹⁵,¹⁸,²¹,²⁵ and in a rat strain congenitally deficient of the precursor kininogen.⁵⁵ Our results also show a diminished thermal hypersensitivity in B2KO mice (table 1). This extends studies in B2KO mice on the 129J genetic background.²¹,²³ For the first time, we analyzed responses in B1KO mice and found no statistically significant difference to WT mice (table 1). Taken together, these results show that kinin receptors are involved in the establishment of thermal hypersensitivity in mild transient inflammation induced by carrageenan.

B1/B2KO were also evaluated in the CFA model of paw inflammation. In comparison with the carrageenan model, CFA induces a stronger (intense paw edema) and more persistent (as long as 3 weeks) inflammation, involving an adaptive immune response to Mycobacterium.⁵⁶-⁵⁷ Our results show that hypersensitivities elicited by intraplantar CFA were equivalent in B1/B2KO and WT mice (fig. 2B). In the CFA model, many inflammatory mediators (cytokines, chemokines) are released from immune cells, and in this context the role of kinins may become relatively negligible. Even if other studies have shown that CFA-induced hypersensitivity is not reduced by the absence or blockade of B2 receptors,²¹,²⁴,⁵⁸ our results were partly unexpected. In fact, several studies described that either B1 antagonist treatment in rats¹⁹,²⁷ or genetic ablation of B1 receptors in mice²⁴,²⁹ is associated with a reduction of thermal and mechanical hypersensitivity in the CFA model. The reasons for these differences are not clear, but because most antagonist studies were performed in rats, it is possible that different mediators are involved in rats and mice. Hypothetical explanations for divergences in B1KO mice studies include differences in doses of Mycobacterium, the assessment of mechanical sensitivities (von Frey filaments vs. algometers²⁵), and genetic backgrounds (C57BL/6 vs. 129J)⁵⁴ because mouse strains differ substantially in nociception.⁵⁹

Peripheral nerve injury has been associated with changes in B1 and B2 receptor expression levels in dorsal root ganglia.⁶⁰,⁶¹ In addition, changes in the cellular populations expressing receptors were found because, after injury, B1 receptors were newly expressed in myelinated dorsal root ganglia neurons and satellite cells.⁶² Surprisingly, our B1/B2KO mice developed normal thermal (heat and cool) and mechanical hypersensitivities after CCI injury, suggesting that kinin receptors do not play a major role in this particular model of peripheral nerve injury at the examined time points. It is still possible that differences could arise in the first minutes or hours after induction of the injury. However, these time points were purposely not tested because they are more related to acute pain caused by surgical operation. Of note, 24 h after CCI injury, hypersensitivities in B1/B2KO were similar to those in WT (data not shown). The lack of effect on tactile and cold hypersensitivities is in accordance with studies using nonpeptidic B1 and B2 antagonists after partial sciatic nerve ligation⁶² or classic B1 and B2 antagonists, des-Arg⁹-[Leu]-bradykinin and HOE140 after spinal nerve ligation⁶³ in rats. Unchanged tactile hypersensitivity was also shown in B1KO mice subjected to partial sciatic nerve ligation or spinal nerve ligation.²⁵,²⁹ However, the influence of kinins on tactile hypersensitivity remains a matter of debate because some studies measured a reduced mechanical hypersensitivity after B1 and B2 blockade in the CCI model in rats³³ and in the partial sciatic nerve ligation model in B1KO mice.³⁰ In terms of heat sensitivity, our findings contrast with reports from other groups in rats²⁵,³¹–³³ and mice.²⁵,³⁰ It is possible that our results are limited to the CCI model in mice because studies in the CCI model have been done only in rats and nerve injuries in B1KO mice were induced by spinal nerve ligation²⁵ and partial sciatic nerve ligation.³⁰ Another explanation could be that the lifelong lack of both kinin receptors leads to adaptive compensatory mechanisms. Additional studies are needed to investigate B1/B2KO mice in models of neuropathic pain in which the B1 receptor was shown to be involved (e.g., partial sciatic nerve ligation, spinal nerve ligation, diabetes).

Our study also shows that in neuropathic B1/B2KO mice, peripheral opioid antinociception induced by the selective µ- and δ-opioid agonists DAMGO and DPDPE was reduced by 30% and 35%, respectively. A large number of studies have shown that peripheral opioid antinociception is detectable or enhanced only under conditions of injury, such as inflammation or neuropathy.⁶⁴ Underlying mechanisms include an increased number and function of opioid receptors in peripheral sensory neurons. The pathogenesis of neuropathic pain states often is influenced by a local inflammation of the injured nerve trunk (for review see Moalem and Tracey⁶⁵). Local inflammatory mediators such as nerve growth factor or interleukin-1β can induce functional competence of opioid receptors.⁶⁶,⁶⁷ Recent in vitro studies show that pretreatment with bradykinin increased the function of µ- and δ-opioid receptors.⁷,⁸ The enhancement of peripheral δ-opioid receptor function also was observed in vivo after priming with bradykinin.⁹ In agreement with the results of most studies in neuropathic pain models in rats,⁶⁸–⁷¹ our results in WT mice show that peripherally acting opioid agonists elicit antinociceptive effects at 2 days after CCI (fig. 3). In B1/B2KO mice, the effects of µ- and δ-opioid agonists were statistically significantly decreased. Thus, although classic effects of kinins are hyperalgesic, our results highlight an involvement in analgesic pathways at least at early time points of nerve injury when inflammation is still present. Whether kinins continue to play a role in opioid antinociceptive effects when chronic pain is well established (weeks after injury) remains to be examined.
In conclusion, the double deficiency of the B1 and B2 receptors showed that kinin receptors are required in models of short-lasting but not of chronic inflammatory and neuropathic pain, and reveal a new protective function of kinins via interactions with opioid receptors in vivo. Therefore, the clinical potential of B1 and B2 antagonists has to be evaluated carefully in terms of antinociceptive potency and efficacy of opioid function.

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ANESTHESIOLOGY REFLECTIONS

Gardner Quincy Colton, a Man of Mark

In 1871 the New York and Hartford Publishing Company released a book of portraits and biographies of "eminent self-made men," a volume titled Sketches of Men of Mark: Written by the Best Talent of the East. The tome was "beautifully illustrated with steel portraits by the finest engravers in the United States." The steel engraving (right) of Gardner Quincy Colton (1814–1898) was accompanied by a six-page biography highlighting Colton’s early efforts as an itinerant lecturer and demonstrator of the recreational use of nitrous oxide. In Hartford, Connecticut, on December 11, 1844, "Dr. Colton took a bag of the gas to the office of Dr. [Horace] Wells and Dr. Colton administered the gas to Wells, when Dr. Riggs [Wells' dental partner] extracted a large molar tooth." As the visionary volunteer, Dr. Horace Wells, regained consciousness, he remarked, "I did not feel it so much as the prick of a pin."

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