Mechanisms Mediating the Vasoactive Effects of the B<sub>1</sub> Receptors of Bradykinin

Irena Duka, Arvi Duka, Ekaterina Kintsurashvili, Conrado Johns, Irene Gavras, Haralambos Gavras

Abstract—Bradykinin normally exerts its vasodilatory effect via the B<sub>2</sub> receptor (B<sub>2</sub>R), but in this receptor’s absence, the B<sub>1</sub> receptor becomes expressed and activated. To explore the mechanism of B<sub>1</sub>R-mediated vasodilation, 8 groups of B<sub>2</sub>R gene–knockout mice received a 2-week infusion of a B<sub>1</sub>R antagonist (300 µg · kg<sup>−1</sup> · d<sup>−1</sup>) or vehicle (groups 1 and 2), B<sub>2</sub>R antagonist or vehicle plus NO inhibition with N<sub>ω</sub>-nitro-L-arginine methyl ester (groups 3 and 4), B<sub>1</sub>R antagonist or vehicle plus cyclooxygenase inhibition with indomethacin (groups 5 and 6), or B<sub>2</sub>R antagonist or vehicle plus blockade of vasoconstricting prostaglandin (PG) H<sub>2</sub> and thromboxane A<sub>2</sub> (TxA<sub>2</sub>) with SQ29548 (groups 7 and 8). The B<sub>1</sub>R antagonist produced significant (P<0.05) blood pressure increases of 17.7±3.1 mm Hg in group 1 and 10.4±3 mm Hg in group 3, whereas their vehicle-treated respective control groups 2 and 4 had no significant blood pressure changes. Indomethacin abolished the capacity of the B<sub>1</sub>R antagonist to raise blood pressure, as did blockade of the receptors of PGH<sub>2</sub> and TxA<sub>2</sub>. Injection with the B<sub>1</sub>R agonist produced a hypotensive response (12±1.3 mm Hg), which was further accentuated by TxA<sub>2</sub> blockade (21.7±4.1 mm Hg). Analysis of B<sub>1</sub>R gene expression by reverse transcription–polymerase chain reaction (PCR) in cardiac and renal tissues revealed marked expression at baseline, with further upregulation by 1.5- to 2-fold after various manipulations. Expression of the TxA<sub>2</sub> receptor gene in renal tissue by quantitative real-time PCR was significantly lower in mice treated with the B<sub>1</sub>R antagonist, consistent with increased levels of agonist for this receptor. The data confirm that the B<sub>1</sub>R becomes markedly expressed in the absence of B<sub>2</sub>R and suggest that it contributes to vasodilation by inhibiting a vasoconstricting product of the arachidonic acid cascade acting via the PGH<sub>2</sub>/TxA<sub>2</sub> receptor. (Hypertension. 2003;42:1021-1025.)

Key Words: receptors, bradykinin ▪ mice ▪ nitric oxide ▪ prostaglandins ▪ vasorelaxation

Bradykinin is a potent vasodilatory nonapeptide<sup>1</sup> that acts via 2 types of receptors: B<sub>1</sub> and B<sub>2</sub>. The B<sub>2</sub> receptors (B<sub>2</sub>Rs) are widely distributed in the vasculature<sup>2</sup> and account for most of its vasodilatory effects. Their activation leads to release of prostaglandins (PGs) and/or nitric oxide (NO),<sup>3-5</sup> whereas their blockade by selective B<sub>2</sub>R antagonists causes a small but significant hypertensive effect.<sup>6</sup>

The B<sub>2</sub>Rs are normally not expressed under physiologic conditions but are inducible by inflammation, lipopolysaccharides, cytokines, vascular trauma, etc.<sup>7-9</sup> Once induced, the B<sub>2</sub>Rs also mediate vasodilation. Indeed, the selective B<sub>2</sub>R agonist des-Arg(9)-bradykinin was shown to reduce blood pressure in rabbits pretreated with lipopolysaccharides, but not in controls,<sup>10</sup> and to relax an in vitro preparation of rabbit mesenteric arteries.<sup>11</sup>

In previous studies, we have shown that the normally nonexpressed B<sub>1</sub>R gene becomes expressed in B<sub>2</sub>R gene–knockout mice, in which the B<sub>2</sub>R assume some of the hemodynamic properties of the B<sub>2</sub>R and is further upregulated in response to hypertensive maneuvers.<sup>12</sup> These findings suggest a role for the B<sub>1</sub>R in vascular tone regulation, but the extent to which these receptors are involved and the mechanism of B<sub>1</sub>R-mediated vasodilation are not clearly understood. The current study was designed to explore this mechanism. For this purpose, we used B<sub>2</sub>R gene–knockout mice (B<sub>2</sub>R<sup>−/−</sup>)<sup>13</sup> and assessed the involvement of NO and/or PGs in B<sub>1</sub>R-mediated vasorelaxation by blocking the synthesis or the receptors of these autacoids and measuring the changes in blood pressure induced by a B<sub>1</sub>R antagonist.

Methods

Animals and Protocols

Eight groups of B<sub>2</sub>R<sup>−/−</sup> mice derived from breeders provided to us by the Jackson Laboratories (Bar Harbor, Me) were used in these experiments.<sup>12</sup> When they were 8 to 12 weeks old and weighed 21 to 31 g, the mice were housed in the animal quarters with a 12-hour light/dark cycle and maintained on a standard chow diet (Purina Rodent Chow 5002) and distilled water ad libitum. All experiments were conducted in accordance with the guidelines for the Care and Use of Animals approved by the Boston University Medical Center.

Group 1 (n=10) received an infusion of a B<sub>1</sub> antagonist, whereas group 2 (n=9) received vehicle solution. Groups 3 and 4 (n=10 each) were given the NO synthase inhibitor N<sub>ω</sub>-nitro-L-arginine methyl ester (L-NAME) in their drinking water. Group 3 received the B<sub>1</sub> antagonist, whereas group 4 served as a control, receiving only vehicle solution. Groups 5 and 6 were treated with subcutaneous...
daily indomethacin; 1 received the B₁ antagonist (n=7), and the other received a vehicle solution (n=9). Groups 7 and 8 (n=10 each) were treated with the thromboxane A₂ (TXA₂/PGF₂α receptor antagonist; SQ29548. Group 7 received the B₁ antagonist, and group 8, a vehicle solution.

All treatments lasted for a 2-week period. Systolic blood pressure (SBP) and heart rate were determined with a noninvasive, computerized, tail-cuff system (BP-2000 Visitech Systems), as described elsewhere. At the end of the experiments, the mice were euthanized by CO₂ inhalation; their hearts and kidneys were removed and prepared for tissue expression of the B₁R gene, as described previously. Two other groups of B₂R−/− mice (n=8 and 7) were anesthetized with pentobarbital (50 mg/kg IP) and had a modified polyethylene catheter introduced into the right iliac artery for direct BP recording, and silicone elastomer (Silastic) tubing was placed in the right iliac vein for drug administration, as described elsewhere. After an overnight recovery period, the arterial line was connected to a BP transducer, and the mean direct BP was recorded with a computerized data-acquisition system (Power Laboratory/400, AD Instruments, Pty Ltd). The baseline BP was recorded for at least 30 minutes or until it became stable. At this point, mice received a 100-μL bolus of a B₁ agonist. When BP returned to baseline, the TXA₂/PGF₂α receptor antagonist or normal saline was infused for a 2-hour period. At this time, a bolus of agonist was infused again, and changes in BP were recorded.

**Expression of B₁R and TXA₂ Receptors in Tissues**

Total RNA was prepared from heart and kidney tissue with TRIzol reagent (GIBCO BRL). The expression of B₁R was examined by reverse transcription–polymerase chain reaction (PCR) techniques, as previously described, whereas the expression of TXA₂ receptors in kidneys was examined by quantitative real-time PCR. Real-time PCRs were performed with an ABI prism 7900 HT sequence detection system with a SYBR Green–based protocol (Applied Biosystems). Oligonucleotide primers for TXA₂ and glyceraldehyde 3-phosphate dehydrogenase (GAPDH, used as a control) were designed with Primer Express Software version 2.0 (Applied Biosystems) and manufactured by Invitrogen and IDT. TXA₂ receptor transcripts were amplified with forward primer AGCACCACGCTCATCTACCC and reverse primer 5′-GACCGGCGAAAGAGGATATAGAC-3′, producing a 34-bp product. GAPDH was amplified with forward primer 5′-TGCACCACCAAATGCTTTAG-3′ and reverse primer 5′-GGATGCAGGATGATGTTCC′, producing a 117-bp product.

The final concentration of the primers for both genes was 300 nmol/L. After an initial denaturation step at 95°C for 10 minutes, the cDNA products were amplified for 40 cycles, consisting of denaturation at 95°C for 15 seconds and a single step of annealing and extension at 60°C for 1 minute. All reactions were run in triplicate and included negative controls. The SDS 2.0 software generated standard curves from 10-fold cDNA dilutions.

**Drugs**

The B₁ antagonist R-892 (AcLys[(oMe)Phe-D-βNal, ile']-des-Arg⁵-bradykinin; a gift from Prof Domenico Regoli, Sherbrooke, Canada) was infused subcutaneously at a dose of 300 μg·kg⁻¹·d⁻¹ through an osmotic minipump (Durect). L-NAME (Sigma) at 50 mg/L was given in the drinking water. Indomethacin (Sigma) at 2 mg·kg⁻¹·d⁻¹ was injected subcutaneously. SQ29548, a TXA₂/PGF₂α antagonist (a gift from Bristol Myers Squibb, Princeton, NJ) was infused subcutaneously at 0.2 mg·kg⁻¹·h⁻¹ through an osmotic minipump. The B₁ agonist des-Arg⁹⁰-Lys-bradykinin (Peninsula Laboratories Inc) was injected intravenously at a dose of 200 μg/kg.

**Statistical Analysis**

All data are expressed as mean±SEM. Two-way ANOVA for repeated measures was used to test for interaction between time and grouping factors. Differences within and between groups were determined by paired and unpaired Student t tests, respectively. A

**Results**

Figure 1A presents the time course of systolic BP in groups 1 and 2, with or without infusion of the B₁ antagonist. In the B₁ antagonist–treated group 1, BP rose from 123.8±1.6 to 141.5±4.2 mm Hg (P<0.05) at the end of the 2-week period. In the vehicle-treated group 2, BP did not increase (124±1.8 vs 125±4.4 mm Hg; P=NS).

Figure 1B shows the time course of SBP in groups 3 and 4 treated with L-NAME, with or without the B₁ antagonist. In group 3, the B₁ antagonist increased the BP from 123.6±1.5 to 133.9±3.2 mm Hg (P<0.05), whereas in group 4, BP did not change significantly from baseline (123.4±1.7 vs 127.9±4.7 mm Hg; P=NS).

Figure 2A shows the time course of SBP in groups 5 and 6 treated with indomethacin, with or without the B₁ antagonist. Neither group increased their BP significantly at the end of the 2-week period: In group5, treated with the B₁ antagonist, BP changed from 113.3±4.01 to 119.4±5.2 mm Hg, and in the vehicle-treated group 6, from 114.1±3.8 to 117.1±3.9 mm Hg (both P=NS).

Figure 2B presents the time course of SBP in the 2 groups of B₁R−/− mice that received treatment with the TXA₂/PGF₂α antagonist SQ29548, with or without the B₁ antagonist. Neither group increased their BP at the end of the 2-week period: In group 7 treated with the B₁ antagonist, BP changed from 125±2.1 to 131±5.3 mm Hg, and in the vehicle-treated group 8, from 126.1±2 to 126.7±7.7 mm Hg.
Addition of the TxA2/PGH2 receptor antagonist as a concurrent infusion for another 120 minutes produced a 25-mm Hg fall in BP from baseline. This sequence is shown in B of Figure 3.

Figure 3 shows the effect of an intravenous injection of the B1 agonist with or without concurrent infusion of the TxA2/PGH2 receptor antagonist (A) or addition of indomethacin (B). Values are mean±SEM. *P<0.05 between the 2 groups.

Figure 2. Time course of SBP in Br−/− mice treated with a B1R antagonist or vehicle alone or with addition of indomethacin (A) or addition of a TxA2/PGH2 receptor antagonist (B). Values are mean±SEM. *P<0.05 between the 2 groups.

There were no differences in body weight among groups at baseline or at end point. B1R mRNA expression was already slightly elevated BP at baseline16 and, consistent with our previous data,12 responded with further BP rises when treated with a B1R antagonist. The present study suggests that NO is not responsible for B1R-mediated vasorelaxation, because the B1R antagonist increased BP to the same extent in the presence or absence of L-NAME, which blocks formation of NO. This is in contrast to other studies in different species, which have shown that stimulation of the B1R with an agonist–

evidence at baseline in the hearts and kidneys of all animals, as shown in the past for Br−/− mice.12 Administration of L-NAME produced no changes in B,R mRNA expression in heart and kidney tissues, as shown in the Table. Indomethacin alone also produced additional increases in B,R mRNA, by 1.5- to 2-fold, in heart and kidney tissues, as did administration of the TxA2/PGH2 receptor antagonist whether given alone or concurrently with the B1 antagonist.

TxA2 receptor expression in kidneys of groups 1 and 2, treated with the B1 antagonist and vehicle, respectively, was determined by quantitative real-time PCR. The slopes of the standard curve for TxA2 and for GAPDH, were -3.28 and -3.4, respectively, where -3.33 corresponds to 100% efficiency of the PCR. The copy numbers for TxA2 were normalized with the data obtained from GAPDH. We found that the expression of TxA2 in mice treated with the B1 antagonist was downregulated by 50% (P<0.05), consistent with increased levels of agonist for these receptors.

Discussion

In previous studies, we have demonstrated that in Br gene–knockout mice, the B1R becomes overexpressed and appears to take over some of the hemodynamic properties of the missing Br.12,20,21 namely, vasorelaxation and contribution to the hypertensive action of angiotensin-converting enzyme inhibitors. The current experiments were designed to explore the mechanism(s) of B1R-mediated vasorelaxation. By evaluating the BP responses to the selective B1R antagonist R89215 administered in Br−/− mice concurrently with inhibition of PG or NO synthesis, we sought to dissect the contribution of each of these factors in mediating the actions of the upregulated B1R. As expected, these mice had a slightly elevated BP at baseline16 and, consistent with our previous data,12 responded with further BP rises when treated with a B1R antagonist. The present study suggests that NO is not responsible for B1R-mediated vasorelaxation, because the B1R antagonist increased BP to the same extent in the presence or absence of L-NAME, which blocks formation of NO. This is in contrast to other studies in different species, which have shown that stimulation of the B1R with an agonist failed to produce vasodilation in pigs or dogs when NO synthesis had also been blocked,17–19 suggesting that those responses were dependent on the release of NO from the endothelium. It has also been suggested that responses to bradykinin can be mediated by the release of other factors, such as an endothelium-derived hyperpolarizing factor or the activation of muscarinic receptors.20,21

On the contrary, inhibition of PG synthesis by indomethacin abolished the hypertensive response to B1R blockade, suggesting that some component of the arachidonic acid cascade is a mediator of this response. There is a body of evidence supporting B1R-mediated vasodilation through PGs, such as in rabbit mesenteric arteries, in coronary arteries of the rat, and in dog mesenteric arteries.11,22,23 This vasodilation can be mediated by synthesis of PGs in the endothelium but might also involve release of endothelium-independent PGs. For example, in some endothelium-denuded vessels, such as in rabbit celiac and mesenteric arteries, activation of the B1R
was reported to result in vasorelaxation that could be inhibited by indomethacin.24,25

The possibility that the B1R might interfere with the synthesis or activity of a vasoconstricting PG has not been considered so far. Our data are consistent with the notion that the B1R interferes with the vasoconstricting influence of some product of the arachidonic acid cascade (eg, TxA2, PGF, or PGH2); blockade of the B1R would release this vasoconstricting product, whereas concurrent blockade of the synthesis of this product or antagonism of this product at the level of its receptor would negate the pressor response to B1R blockade). Indomethacin blocks cyclooxygenase and production of all downstream PGs, both the vasodilating ones as well as vasoconstricting ones, like PGH2 and Tx. In our last 2 experimental groups, we decided to selectively block the receptors to these vasoconstricting products, ie, Tx and its precursor PGH2, which share the same receptor, with the compound SQ29548. The fact that this compound prevented the expected rise after B1R blockade would suggest that B1R activity might be suppressing these vasoconstricting substances. To further explore this hypothesis, a B1 agonist was injected before and after TxA2/PGH2 blockade. The hypotensive effect of the B1 agonist was increased after TxA2/PGH2 blockade. Expression of the TxA2 receptor gene, assessed by real-time PCR in kidneys of mice treated with a B1 antagonist, showed significant downregulation of the TxA2 receptor gene, probably as a compensatory mechanism resulting from an increase in TxA2 levels after B1R blockade.

Thus, our current data lead to the hypothesis of a possible dissociation between PG-mediated and NO-mediated vascular effects, so that vasorelaxation resulting from stimulation of B1Rs need not be NO mediated but rather might be attributable to B1R-mediated suppression of a vasoconstricting component of the arachidonic acid cascade. The accen-

<table>
<thead>
<tr>
<th>Group</th>
<th>Kidney</th>
<th>Heart</th>
</tr>
</thead>
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<tr>
<td>Untreated</td>
<td>0.97±0.04</td>
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<tr>
<td>L-NAME+B1A</td>
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<td>Indomethacin+B1A</td>
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<td>1.25±0.25</td>
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<tr>
<td>Indomethacin</td>
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<td>1.72±0.05*</td>
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<tr>
<td>SQ29548+B1A</td>
<td>1.4±0.06*</td>
<td>2±0.05*</td>
</tr>
<tr>
<td>SQ29548</td>
<td>1.62±0.18*</td>
<td>1.5±0.18*</td>
</tr>
</tbody>
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B1A indicates B1 antagonist. Values are mean±SEM derived from 4 mice in each group. Mean densitometric data are expressed as a ratio of 18S mRNA, derived from reverse transcription-PCR.

*P<0.05 between treated and untreated mice.

Variations of mRNA Coding for B1Rs in Kidneys and Hearts of B1R/-/- Mice as a Function of Various Treatments
uated hypotensive response to a B1R agonist under concurrent blockade of the TxA2/PGH2 receptor further corroborates this interpretation.

The discrepancies between different studies indicate that the mechanism of B1R-mediated vasodilation is heterogeneous and might be species specific as well as vessel specific. The different experimental settings might also account for the differences between studies, as our study was conducted in vivo in conscious mice with a genetically deleted B2R, whereas others were conducted in normal animals with various pharmacologic blockers or in vitro in isolated blood vessels. These experimental inconsistencies illustrate the complexity of the mechanisms involved in B1R-mediated vasodilation.

In addition to the BP response, there was an upregulation of B1R in kidney and heart tissues, in keeping with our previous findings in B1R−/− subtotally nephrectomized mice. This upregulation is accentuated by hypertensive manipulations and might contribute to the lack of a significant BP rise in response to such manipulations in these animals with normal renal function. This is in contrast to the significant loss of insulin sensitivity in B2R−/− animals with normal renal function. This is in contrast to the previous findings in B2R−/− mice. 12 This upregulation is accentuated by hypertensive manipulations and might contribute to the lack of a significant BP rise in response to such manipulations in these animals with normal renal function. This is in contrast to the significant loss of insulin sensitivity in B2R−/− mice, in which B1R upregulation cannot compensate for this property. 26

**Perspectives**

The current findings corroborate and amplify previous data indicating that in the absence of the B2R, bradykinin can still exert a tonic vasorelaxant effect via activation of B1Rs, as shown by the hypertensive response to B2R blockade. Preservation of the hemodynamic properties of bradykinin via the B1R in the absence of a B2R is of particular interest when considered in light of the fact that its metabolic (insulin-sensitizing) properties seem to be solely dependent on the B2R and are lost in its absence. The vasorelaxant effect of the B1R does not seem to be effected via NO release, because inhibition of NO synthesis by L-NAME did not accentuate the hypertensive response to B2R blockade. The fact that indomethacin did abolish this response would suggest that some component of the arachidonic acid cascade might be involved in the B1R-mediated vascular effects; this compound would have to be a vasoconstricting substance that is suppressed by B1R activity and becomes apparent after B2R blockade. The fact that TXA2/PGH2 receptor inhibition prevented the BP rise expected after B2R blockade would corroborate this interpretation. Further elucidation of the complex relation between vasoconstricting and vasodilatory factors and their metabolic functions should enhance the tissue-protective efficacy of interventions aimed at lowering BP to improve perfusion and preserve nutrition of vital organs.

**Acknowledgments**

This study was supported in part by National Institutes of Health grants RO1 HL 58807 and P50 HL 55001.

**References**


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Hypertension. 2003;42:1021-1025; originally published online October 13, 2003;
doi: 10.1161/01.HYP.0000097550.98865.35
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/42/5/1021

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