Role of bradykinin B1 and B2 receptors in normal blood pressure regulation

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Duka, Arvi, Irena Duka, Guohong Gao, Sherene Shenouda, Irene Gavras, and Haralambos Gavras. Role of bradykinin B1 and B2 receptors in normal blood pressure regulation. Am J Physiol Endocrinol Metab 291: E268–E274, 2006. First published February 28, 2006; doi:10.1152/ajpendo.00382.2005.—With inhibition or absence of the bradykinin B2 receptor (B2R), B1R is upregulated and assumes some of the hemodynamic properties of B2R, indicating that both participate in the maintenance of normal vasoregulation or to development of hypertension. Herein we further evaluate the role of bradykinin in normal blood pressure (BP) regulation and its relationship with other vasoactive factors by selectively blocking its receptors. Six groups of Wistar rats were treated for 3 wk: one control group with vehicle alone, one with concurrent administration of B1R antagonist R-954 (70 μg·kg−1·day−1) and B2R antagonist HOE-140 (500 μg·kg−1·day−1), one with R-954 alone, one with HOE 140 alone, one with concurrent administration of both R-954 and HOE-140 plus the angiotensin antagonist losartan (5 mg·kg−1·day−1), and one with only losartan. BP was measured continuously by radiotelemetry. Only combined administration of B1R and B2R antagonists produced a significant BP increase from a baseline of 107–119 mmHg at end point, which could be partly prevented by losartan and was not associated with change in catecholamines, suggesting no involvement of the sympathoadrenal system. The impact of blockade of bradykinin on other vasoregulating systems was assessed by evaluating gene expression of different vasoactive factors. There was upregulation of the eNOS, AT1 receptor, PGE2 receptor, and tissue kallikrein genes in cardiac and renal tissues, more pronounced when both bradykinin receptors were blocked; significant downregulation of AT2 receptor gene in renal tissues only; and no consistent changes in B1R and B2R genes in either tissue. The results indicate that both B1R and B2R contribute to the maintenance of normal BP, but one can compensate for inhibition of the other, and the chronic inhibition of both leads to significant upregulation in the genes of related vasoactive systems.

bradykinin inhibition; angiotensin II; vasoactive factors; gene expression

DESPITE A LARGE BODY OF LITERATURE on kinins, the role of bradykinin in normal blood pressure (BP) regulation and the specific functions mediated by its B1 and B2 type receptors are still insufficiently understood.

It has long been accepted that under normal conditions the cardiovascular and renal effects of bradykinin are exerted via the B2 receptors, which are constitutively abundant in cardiac, renal, and vascular tissues and act by activating the vasoactive components of the prostaglandin-NO cascade (34), whereas the B1 receptors are inducible by stimuli such as tissue damage, bacterial lipopolysaccharides, etc., and are mostly involved in inflammatory and nociceptive reactions (35, 28).

Pharmacological experiments using selective antagonists of the B2 receptor in normotensive rats have given contradictory results: whereas some demonstrated an acute or chronic increase in BP (7, 24), others failed to show change in normal BP (3, 23), although they did show exacerbation of salt-induced (25) or angiotensin-induced hypertension (26) during B2 receptor blockade. The issue was further complicated when it became apparent that some of these bradykinin analogs could cause sympathoadrenal stimulation similar to that elicited by bradykinin itself (31).

The availability of genetically engineered mice with deletion of one or the other of these receptors (8, 33) permitted further elucidation of their vascular effects: mice with deleted B2 receptor gene are already mildly hypertensive (12, 14, 27) and exhibit an exaggerated hypertensive response to high-salt diet (1), DOCA salt-induced hypertension (15), and exogenous angiotensin II infusion (27) but, surprisingly, not to endogenous angiotensin II stimulation resulting from renal artery clipping (12). Further investigation of the last model suggested that B2 receptor gene knockout mice displayed a striking upregulation of the B1 receptor gene, which was further enhanced by experimental manipulations (12) and could assume some of the hemodynamic properties of the B2 receptor by activating components of the arachidonic acid cascade (14), although it did not replace the insulin-sensitizing properties of the B2 receptor (13).

However, bradykinin is known to interact with a variety of local humoral factors (10), and the deletion of a gene may well entail compensatory changes during ontogenesis in other vasoactive factors, whose altered status contributes to the phenotype of the genetically engineered animal. In such case, any alterations in vasoregulatory systems may not be representative of physiological status. By contrast, chronic inhibition of a particular factor in the adult intact animal should be more representative of the consequences of inactivation of this factor and, hence, provide more accurate information about its properties and its interaction to other factors relevant to its function. The purpose of the present experiments was to further investigate the vasoregulatory effects of the B1 and B2 receptors of bradykinin in normal rats by chronically inhibiting each one separately or both concurrently and assessing the results on blood pressure and on gene expression of related vasoactive factors evaluated by quantitative real-time reverse transcription-polymerase chain reaction (QRT-PCR).

METHODS

Animals and Procedures

Male Wistar rats weighing 270–350 g (Charles River Laboratories, Wilmington, MA), were used in these experiments, which were conducted in accordance with the guidelines for the Care and Use of Animals approved by the Boston University Medical Center. For BP...
measurements, a PA-C40 radiotelemetry BP transmitter probe (Data Sciences International) was implanted in the abdominal aorta. After a 5- to 7-day recovery period, baseline BP was recorded for 3 days. Starting on the 4th day, the various pharmacological probes were administered subcutaneously for a period of 3 wk using Alzet osmotic pumps (DURECT, Cupertino, CA) implanted dorsolaterally. We used six groups of animals for this experiment. Group 1 (n = 8) received vehicle solution (saline); group 2 (n = 8) received concurrently a B1 receptor antagonist (R-954; 70 μg·kg⁻¹·day⁻¹) and a B2 receptor antagonist (HOE-140; 500 μg·kg⁻¹·day⁻¹); group 3 (n = 7) received only the B1 receptor antagonist; group 4 (n = 7) received only the B2 receptor antagonist; group 5 (n = 10) received concurrently both bradykinin receptor antagonists along with the angiotensin II antagonist losartan (5 mg·kg⁻¹·day⁻¹) and group 6 (n = 8) received only the angiotensin II antagonist losartan. BP was constantly monitored by radiotelemetry.

The B1 receptor antagonist R-954 was a gift from Dr. F. Gobeil, Jr. and was chosen for its potency and resistance to enzymatic degradation (32). The B2 receptor antagonist HOE-140 (Icaltiban; Sigma-Aldrich, Milwaukee, WI) has been used extensively by us and others in animal and human studies.

At the end of the experiments, rats were euthanized with CO2, their hearts and kidneys harvested, placed in solution of RNAlater (Ambion, Austin, TX) and frozen at −80°C. Total RNA was extracted using TRIzol (Invitrogen, Carlsbad, CA), according to the manufacturer’s specifications.

**Quantitative Real-Time RT-PCR**

The TaqMan reverse transcription reagents (Applied Biosystems, Foster City, CA) were used to synthesize cDNA in a 50-μl reaction containing 1 μg of DNase I-treated (Ambion) total RNA. The RT reaction was carried out as suggested by the manufacturer at 25°C for 10 min, 48°C for 30 min, and 95°C for 5 min. The cDNA was analyzed immediately or stored at −20°C for later use. QRT-PCR reactions were performed with the ABI Prism 7900HT Sequence Detection System using a SYBR Green-based protocol (Applied Biosystems). The total volume of the real-time PCR reactions was 20 μl, and the amount of cDNA that was used per reaction was ~0.04 μg. Oligonucleotide primers for RT-PCR were designed with the Primer Express 2.0 software program (Applied Biosystems) and manufactured by Integrated DNA Technologies (Coralville, IA). To ascertain the specificity of the amplicons, a BLASTN analysis was done, and an additional step, the dissociation stage, was performed at the end of every real-time PCR experiment according to the manufacturer. The sequences of the primers used in expression analysis are shown in Table 1. All reactions were run in triplicate and included negative controls. The concentrations of the forward and reverse primers were ~300 nM. After initial denaturation at 95°C for 10 min, the cDNA products were amplified for 40 cycles consisting of denaturation at 95°C for 15 s and annealing and extension performed in a single step at 60°C for 1 min. The SDS 2.1 software-generated standard curves from 10-fold serial cDNA dilutions and the threshold cycle were normalized for each standard curve. The slopes were between −2.94 and −3.44, where −3.33 corresponds to 100% efficiency of the PCR reaction. The copy numbers for all samples were normalized with the data obtained from GAPDH endogenous controls.

**Determination of Plasma Catecholamine Levels**

At the end of the experiment in groups 1 and 2, the iliac artery was catheterized for blood drawing. On the day after catheterization, with the animals conscious and quiet, 100 μl of blood were drawn slowly from the arterial line into a syringe presyrined with EGTA (90 mg/ml) and reduced glutathione (60 mg/ml) solution RPN532 (Amersham Life Science), which was used as an anticoagulant and antioxidant. The blood was expelled through the needle into an Eppendorf tube, and plasma was separated by spinning at 900–1,000 g in a variable-speed centrifuge. The plasma was transferred to fresh tubes, sealed, and stored at −80°C until assay. Rat plasma (10–20 μl) was diluted to 50 μl with sterile water to produce the 50-μl volume needed in the assay. Plasma norepinephrine and epinephrine were measured by the BioTrak Catecholamine Research Assay System TRC 995 (Amersham Life Science). The assay is sensitive to ~2 pg of norepinephrine or epinephrine per tube.

**Statistical Analysis**

**Gene expression.** Statistical analysis was performed by Student’s t-test. A P value of ≤0.05 was considered statistically significant. All experiments were performed two or three times to ascertain reproducibility.

**Blood pressure.** All data are expressed as means ± SE. Two-way ANOVA for repeated measures was used to test for interaction between time and grouping factor. Differences within and between groups were determined using paired and unpaired Student’s t-tests, respectively. Differences at P ≤ 0.05 were considered significant.

**RESULTS**

**Blood Pressure Measurements**

Only the group submitted to concurrent blockade of both B1 and B2 receptors exhibited a significant increase in BP by 12 ± 2 mmHg (P < 0.05). The infusion of losartan produced a significant decrease in BP by 13.8 ± 2.3 (P < 0.05) when administered alone and attenuated the increase in BP when administered concurrently with the B1 and B2 receptor blockers. All other groups had minor changes ranging between 4 and 6 mmHg [P = not significant (NS)]. The daily BPs of each group compared with the saline-infused controls are shown in the five figures. Figure 1 shows that combined infusion of both bradykinin receptor antagonists produced a rise in BP that
became significant by day 13 and was significantly different from the control group during the last 3 days. Figure 2 shows no change in BP in the B1 receptor antagonist-infused animals (Δ = 2.3 ± 0.5) and no difference from controls. Likewise, Fig. 3 shows no change in BP in the B2 receptor antagonist-infused animals (Δ = 6 ± 1.5) and no difference from controls. Figure 4 shows a significant fall of BP upon initiation of infusion of the two bradykinin antagonists combined with losartan but no further change and overall no difference in BP (Δ = 4.2 ± 1.1) from controls. Figure 5 shows a significant fall of BP upon initiation of infusion of losartan, which was maintained to the end of the 3-wk period.

**Gene Expression Analysis**

To explore the impact of blockade of bradykinin effects on other systems, we analyzed in cardiac and renal tissues the gene expression of the following vasoactive factors: angiotensin II receptor type 1 (AT1R), endothelial nitric oxide synthase (eNOS), prostaglandin E2 receptor (PGE2R), tissue kallikrein (KLK), angiotensin II receptor type 2 (AT2R), bradykinin B1 receptor, and bradykinin B2 receptor. The results are shown in Table 2.

It is apparent that the eNOS gene expression increased significantly in both organs with all manipulations. The AT1R gene expression likewise increased with all manipulations in kidney tissues, but in the heart it increased only when both bradykinin receptors were blocked. The PGE2R gene expression remained unchanged in the heart but increased in the kidney with all manipulations. The KLK gene expression also remained unchanged in cardiac tissues, but in renal tissues it increased only with the combined bradykinin antagonist infusion and was unaffected by losartan. The AT2R gene was significantly decreased in the renal tissues in all manipulations, whereas it was very minimally expressed in the cardiac tissue. Bradykinin B1 and B2 receptor genes displayed no consistent changes in either cardiac or renal tissues.
Catecholamine Measurements

At end point, there was no difference between control rats (group 1) and the rats that received concurrently both bradykinin receptor antagonists (group 2) in plasma levels of nor-epinephrine (0.28 ± 0.05 vs. 0.35 ± 0.04 ng/ml, \( P = \text{NS} \)) or epinephrine (0.11 ± 0.02 vs. 0.22 ± 0.06 ng/ml, \( P = \text{NS} \)).

DISCUSSION

These studies indicate that inhibition of either the B1 or the B2 receptor of bradykinin alone in adult normotensive rats produces no change in systemic BP over a period of 3 wk. This finding is consistent with most reports in the literature, as mentioned earlier (3, 23, 28, 31, 34, 35). It appears that the B2 receptor-mediated vasodilatory action of bradykinin is important for the perfusion of certain regions, such as the papillary region of the kidney (16) or the subendocardial region of the myocardium (36), but has no major impact on systemic hemodynamics. This lack of systemic hypertensive response to inactivation of the B2 receptor is partly due to the fact that elimination of the B2 receptor results in upregulation of the B1 receptor (14), which is also capable of activating the prostaglandin-NO cascade (14), thus preventing the rise of vascular resistance. This is further supported by the fact that concurrent inhibition of both receptors is necessary to produce a rise in systemic BP, as shown in the present experiments, as well as in previous studies in B2 receptor gene knockout mice, in which B1 receptor blockade produced a significant hypertensive response (14). It should be noted, however, that, in the B2 receptor gene knockout mice, the baseline BP is already slightly but significantly higher than in their wild-type counterparts (12, 14, 27), even though pharmacological B2 receptor blockade failed to raise BP (3, 23).

Of particular interest in our current experiments was the fact that, in these normal adult rats, the hypertensive response to concurrent blockade of both bradykinin receptors did not occur...
Gene expression results

Table 2.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Heart</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>eNOS</td>
<td>AT1R</td>
<td>PGE2R</td>
</tr>
<tr>
<td>B1R + B2R antag vs. saline</td>
<td>3.78±0.54*</td>
<td>3.67±0.03*</td>
</tr>
<tr>
<td>B1R antag vs. saline</td>
<td>3.61±0.5*</td>
<td>no change</td>
</tr>
<tr>
<td>B1R antag vs. saline</td>
<td>5.38±1.60*</td>
<td>no change</td>
</tr>
<tr>
<td>B1R + B2R antag + Los vs. saline</td>
<td>9.96±1.41*</td>
<td>4.35±2.08</td>
</tr>
<tr>
<td>Los vs. saline</td>
<td>1.78±1.41*</td>
<td>no change</td>
</tr>
<tr>
<td>B1R + B2R antag vs. saline</td>
<td>1.52±0.11*</td>
<td>1.94±0.06*</td>
</tr>
<tr>
<td>B1R antag vs. saline</td>
<td>1.61±0.06*</td>
<td>2.67±0.03*</td>
</tr>
<tr>
<td>B1R antag vs. saline</td>
<td>1.81±0.14*</td>
<td>2.95±0.3*</td>
</tr>
<tr>
<td>B1R + B2R antag + Los vs. saline</td>
<td>2.16±0.35*</td>
<td>10±3.25</td>
</tr>
<tr>
<td>Los vs. saline</td>
<td>2.56±0.64*</td>
<td>2.59±0.72</td>
</tr>
</tbody>
</table>

Los, losartan; antag, antagonist. Numbers represent the fold difference in expression level of genes in heart and kidney tissue in experimental (n = 5 each) relative to saline control (n = 5) animals and are expressed as means ± SE. *P < 0.05.
cascade (4, 5, 19); some of these are also involved in the natriuretic function of bradykinin, and this is particularly true for PGE₂ (2, 22). The interaction between these factors is illustrated by the significant upregulation of the PGE₂R gene in renal tissues in the current experiments. Likewise, the close interaction between bradykinin and angiotensin II is illustrated where they occurred with all bradykinin-blocking maneuvers. A close interaction between these two systems has been reported in the past; indeed, part of the BP-lowering effect of AT₁R blockade has been attributed to kinin stimulation via the activated AT₂R (37).

In summary, these studies indicate that, under normal conditions, the vasoregulatory properties of bradykinin are mediated mostly via its B₂ receptor but that when this receptor is inactivated they can be carried out by upregulation of the B₁ receptor. In genetically engineered mice in the past, this receptor was shown to be capable of activating the arachidonic acid cascade (13, 21). Furthermore, the present studies indicate that disturbance of the vasoregulatory equilibrium by chronic interference with one vasoreactive substance leads to changes in gene expression of numerous other substances related to BP regulation and tissue perfusion.

GRANTS
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REFERENCES


