Role of the B₂ Receptor of Bradykinin in Insulin Sensitivity

Irena Duka, Sherene Shenouda, Conrado Johns, Ekaterina Kintsurashvili, Irene Gavras, Haralampos Gavras

Abstract—The biological actions of bradykinin (BK) are attributed to its B₂ type receptor (B₂R), whereas the B₁R is constitutively absent, inducible by inflammation and toxins. Previous studies in B₂R gene knockout mice showed that the B₁R is overexpressed, is further upregulated by hypertensive maneuvers, and assumes some of the hemodynamic functions of the B₂R. The current experiments were designed to further clarify the metabolic function of the B₂R and to explore whether the upregulated B₁R can also assume the metabolic function of the missing B₂R. One group of B₂R−/− mice (n=9) and one of B₂R+/+ controls (n=8) were treated for 3 days with captopril (which produced a similar blood pressure–lowering response in both groups) and studied with the hyperinsulinenemic euglycemic clamp. The knockout mice had fasting and steady-state blood glucose levels similar to those of the wild-type mice but a had tendency to higher fasting insulin levels (at 27.8±5.2 versus 18±2.9 mU/L, respectively). However, they had significantly higher steady-state insulin levels (749±127.2 versus 429.1±31.5 mU/L, P<0.05) and a significantly lower glucose uptake rate (31±2.4 versus 41±2.3 mg/kg per minute, P<0.05) and insulin sensitivity index (4.6±0.9 versus 10±0.7 P<0.001). Analysis of B₂R and B₁R gene expression by reverse transcription–polymerase chain reaction in cardiac muscle, skeletal muscle, and adipose tissues revealed significantly higher B₁R mRNA level in the knockouts versus wild-type (P<0.05) at baseline and a further significant upregulation in mRNA by 1.8- to 3.2-fold (P<0.05) after insulin infusion. We conclude that absence of B₂R confers a state of insulin resistance because it results in impaired insulin-dependent glucose transport; this is probably a direct B₁R effect because, unlike the hemodynamic autacoid-mediated effects, it cannot be assumed by the upregulated B₁R. (Hypertension. 2001;38:1355-1360.)

Key Words: insulin • hyperinsulinism • mice • bradykinin • gene expression

Bradykinin mediates a variety of biological effects such as vasodilation, vascular permeability, inflammation, pain, and edema.¹ It is also known to play an important role in glucose metabolism.² ³ Indeed, it was shown in vitro and in vivo that administration of bradykinin increases the glucose uptake in cultured adipocytes⁴ as well as in long-term rat experiments⁵ and in skeletal muscle of human forearm.⁶ ACE inhibitors were shown to improve glucose utilization,⁷ an action that is attributed to bradykinin.⁸ ⁹ In keeping with these data, kinogen-deficient rats were found to be resistant to insulin.¹⁰

The effects of bradykinin are mediated by the B₁- or B₂-type receptor (B₁R or B₂R). It has been accepted that almost all of the physiologically significant effects of bradykinin, including the metabolic ones, are exerted by activation of the B₂R. Indeed, inhibition of the B₁R by various antagonists was shown to reverse the amelioration of insulin-dependent glucose transport by ACE inhibitors,¹¹ ¹² whereas blockade of downstream mediators, such as prostaglandins and NO, had no effect on insulin sensitivity.¹² Several studies have shown that the B₁R is expressed in tissues dependent on insulin for glucose uptake, such as skeletal muscle and adipocytes.⁴ ¹³ ¹⁴ On the contrary, the B₂R is not expressed under normal conditions; it has long been known that its expression is induced by toxins or inflammatory mediators and it contributes to endotoxic shock,¹⁵ but it has not been associated with metabolic functions.

In a recent series of studies, investigators have used genetically engineered mice with deleted B₂R¹⁶ to further explore the physiological actions of bradykinin. Using these mice, we observed that the B₂R is highly expressed in B₂R knockout mice and appears to take over some of the hemodynamic properties of the B₂R.¹⁶ The present experiments were designed to further explore the metabolic function of the B₂R and to investigate whether in the absence of B₂R, the upregulated B₁R might also be able to take over the metabolic functions of bradykinin. To this aim, we evaluated the differences in insulin sensitivity in B₂R knockout mice and their wild-type controls, by using the hyperinsulinenemic euglycemic clamp technique.

Methods

Animals

Bradykinin B₂R gene knockout mice (B₂R−/−)¹⁶ and their wild-type B6 129SvF2 controls (B₂R+/+), obtained from the Jackson Labo-
Euglycemic Hyperinsulinemic Clamp Procedure

Under anesthesia with pentobarbital (50 mg/kg IP), two catheters were inserted in the left jugular vein for infusion of glucose and insulin and one in the iliac artery for blood sampling. The mice were maintained supine throughout the 2-hour duration of the procedure on a heating pad at 37°C. Anesthesia was confirmed by the absence of corneal and toe-pinch reflexes. Porcine insulin (Eli Lilly & Co) was infused at a rate 12 mU/kg per minute, and 5% glucose was infused through a Harvard pump (Harvard Apparatus Inc) at variable rates as needed to maintain euglycemia. Blood samples (10 μL) were collected every 5 to 10 minutes from the iliac artery and assayed by the Accu-Chek II glucose monitor; 250 μL of blood was withdrawn to measure plasma insulin levels at time zero and at the end of the hyperinsulinemic infusion. A donor mouse was used to replace the blood withdrawn at the beginning of the experiment. Plasma insulin levels were measured by a rat immunoassay kit (Linco Research).

The following parameters were recorded: (1) fasting plasma glucose and insulin levels, (2) plasma glucose levels at each of the 6 to 8 samplings throughout the clamp period, (3) steady-state plasma glucose levels as reflected by the mean of the plasma glucose levels in the last 30 minutes of the clamp, (4) plasma insulin level at the end of the clamp, and (5) glucose uptake as reflected by the mean glucose levels at time zero and at the end of the hyperinsulinemic clamp. The data were presented in Figure 1. The Table shows blood glucose and insulin parameters during the hyperinsulinemic euglycemic clamp procedure.

Expression of Bradykinin Receptors in Tissues

Total RNA was prepared from heart, skeletal muscle, and adipose tissue, with TRIzol Reagent (GIBCO BRL). A DNase digestion step was performed to increase the purity of the RNA samples with total RNA Isolation Kit S.N.A.P. (Invitrogen). The expression of B1R and B2R in the heart, skeletal muscle, and adipose tissues was examined by reverse transcription–polymerase chain reaction (RT-PCR) techniques as previously described.17

Statistical Analysis

All data are expressed as mean±SEM. Student’s t tests for paired and unpaired data were used as appropriate. The Mann-Whitney rank sum test was used for nonparametric data. Differences at P<0.05 were considered significant.

Results

SBP at baseline and after treatment with captopril are shown in Figure 1. Baseline SBP was higher in knockout mice than in their wild-type controls (121.6±2.94 versus 109.8±1.78 mm Hg, P<0.05). After 3 days of captopril treatment, the SBP decreased significantly in both groups (to 87.5±2.33 mm Hg in the B2R−/− and 91.6±2.41 mm Hg in the wild-type mice), with no difference between the two groups at end point.

The Table shows blood glucose and insulin parameters during the hyperinsulinemic euglycemic clamp procedure.
insulin-dependent glucose transport. Although studies without ACE inhibition would better reflect physiological conditions, we chose to use pretreatment with ACE inhibition to maximize the influence of endogenous bradykinin. We found that animals lacking the B₂R had a similar BP response to the ACE inhibitor as the wild-type animals but exhibited relative insulin resistance, as shown by tendency to elevated fasting insulin levels that were further exaggerated during hyperinsulinemic conditions under ACE inhibition. Although elevated insulin levels could be attributable to reduced insulin clearance, their presence in the face of normoglycemia is

### Parameters of Glucose Metabolism in B₂R−/− Mice and Wild-Type Counterparts During Hyperinsulinemic Euglycemic Clamp

<table>
<thead>
<tr>
<th>Group</th>
<th>Fasting Glucose, mg/dL</th>
<th>Steady-State Glucose, mg/dL</th>
<th>Glucose Uptake, mg/kg per min</th>
<th>Fasting Insulin, mU/L</th>
<th>Steady-State Insulin, mU/L</th>
<th>Insulin Sensitivity Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>B₂R+/+ (n=8)</td>
<td>75.6±5.3</td>
<td>103.1±2.9</td>
<td>41±2.3</td>
<td>18.0±2.9</td>
<td>429.1±31.5</td>
<td>10±0.7</td>
</tr>
<tr>
<td>B₂R−/− (n=9)</td>
<td>85.8±5.6</td>
<td>105.8±1.7</td>
<td>31±2.4*</td>
<td>27.8±5.2</td>
<td>749±127.2*</td>
<td>4.6±0.9†</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SEM. *P<0.05; †P<0.001 between knockout and wild-type mice.

---

**Figure 2.** Analysis of B₁R mRNA (A) and B₂R mRNA (B) in skeletal muscle at baseline (captopril treated) and end point (captopril treated, after hyperinsulinemic euglycemic clamp) in B₂R−/− and B₂R+/+ mice. Top, Representative RT-PCR; bottom, mean densitometric data expressed as ratio of 18S mRNA (n=3 in each group). *P<0.05 between knockout and wild-type mice, †P<0.05 between baseline and end point.

**Figure 3.** Analysis of B₁R mRNA (A) and B₂R mRNA (B) in adipose tissue at baseline (captopril treated) and end point (captopril treated, after hyperinsulinemic euglycemic clamp) in B₂R−/− and B₂R+/+ mice. Top, Representative RT-PCR; bottom, mean densitometric data expressed as a ratio of 18S mRNA (n=3 in each group). #P<0.05 between baseline and end point.
characteristic of diminished tissue sensitivity to insulin. Glucose uptake was reduced by 25% and the insulin sensitivity index by 54% compared with the wild-type mice. The data indicate that absence of B2 R causes a state of insulin resistance, as it is associated with impaired glucose metabolism. This is in agreement with previous data, which have shown that selective B2 R antagonists but not by the prostaglandin inhibitor indomethacin or the NO synthase inhibitor nitro-L-arginine methyl ester (L-NAME). Although other investigators have corroborated these findings, there is still no unanimous agreement as to the role of local tissue mediators in this aspect of the B2 R function. Nevertheless, it is possible that alterations in number or function of B2 R may contribute to the diminished insulin sensitivity encountered in conditions, such as normal aging or essential hypertension.

In a recent study, we found that the B1 R, which is physiologically inert, was actually overexpressed in B2 R knockout mice at baseline and was further upregulated during hypertensive procedures. Furthermore, under those conditions it appeared to take over part of the hemodynamic effect of the missing B2 R. This would explain why the B2 R knockout mice have no lesser antihypertensive response to ACE inhibition than the wild-type, as described several years ago. These findings are consistent with the BP results of the current study, in which the BP response to ACE inhibition remained unaffected. The cardiovascular phenotype of B2 R knockout mice is a matter of controversy in the literature, as some authors have found them to be normotensive at baseline whereas others have found them to be hypertensive, which is in agreement with our own current and previous data. These discrepancies are difficult to explain because they may reflect not only the genetically engineered mutation but also selection, genetic drift, and epistatic interactions that may occur in small-size colonies.

Regardless of cardiovascular phenotype, the current study was designed to further clarify the metabolic role of the B2 R and to explore whether the upregulated B1 R may also be capable of taking over part of the metabolic properties of bradykinin. Additional studies using specific B1 R antagonists would, of course, further corroborate or refute these data, because it is possible that resistance to insulin might become further accentuated during B1 R blockade. Nevertheless, the increased insulin resistance in the B2 R knockout mice suggests that the upregulated B2 R receptor does not take over the metabolic function of the missing B2 R. This evidence may also be taken to further support the notion that unlike the hemodynamic action, the metabolic action is a direct effect of the B2 R, not mediated by downstream autacoids such as NO or prostaglandins, which may respond to stimulation by other receptors.
One could offer possible speculative explanations for the failure of the B1R to exert direct effects, for example, failure to internalize.

In wild-type mice, analysis of the mRNA expression of the B1R and B2R in skeletal muscle, heart, and adipose tissues, that is, tissues that are most dependent on insulin for glucose uptake, revealed that the insulin infusion induced also an upregulation of B2R. This receptor is constitutively present, and its upregulation by this maneuver is a novel finding. This is consistent with the notion that this bradykinin receptor plays an important role in insulin-mediated glucose transport in skeletal muscle, adipose tissue, and heart, although differences in degree of overexpression among tissues are difficult to explain. An unexpected finding, however, was that along with the B2R upregulation, there was also in these animals an increase in B1R gene expression, although to a much lesser extent than the B2R and to degrees varying widely among different tissues. As mentioned earlier, it is known that the B1R is not expressed under physiological conditions, but pathological conditions such as inflammation and tissue damage induce its expression in vascular and nonvascular tissues.15 The inducibility of this receptor was first described in isolated rabbit aorta, in which a time-dependent and protein synthesis–dependent contractile response to des-Arg-BK was observed.34,35 It was recently shown that the BK B2R upregulation involves activation of protein kinases through participation of nuclear factor-xB, identified in the promoter region of the B2R gene.36–38 Insulin receptor activation also involves mobilization of tyrosine kinase,39 similar to the mobilization induced by cytokines, which may explain the inducibility of B1R by insulin. The functional significance, if any, of this phenomenon, remains unclear at this time. Usually, biologic responses have a teleologically plausible, even if speculative, phenotype, remains unclear at this time. Usually, biologic responses have a teleologically plausible, even if speculative, justification. Accordingly, it is easy to explain why deletion of B2R would elicit induction of B1R, which is also capable of stimulating release of local tissue mediators such as NO and prostaglandins40 and hence maintain local tissue perfusion. Likewise, it is easy to see why infusion of insulin and glucose would upgrade expression of the B1R; however, a change in transcriptional regulation of the B2R gene by this maneuver, especially in the presence of a normally functioning upgraded B2R, is difficult to explain away when the B1R can have no impact on glucose metabolism. Of course, it should also be kept in mind that increased B2R mRNA does not necessarily imply increased generation of B2R protein, although it is strongly suggestive.

Other pathological conditions, in which upregulation of the B2R has been described in various tissues, include regional ischemia41 and hyperglycemia caused by diabetes mellitus induced by streptozotocin.42,43 All of these experiments, however, have only explored the hemodynamic functions assumed by the B2R, which become activated even in the presence of functional B1R and contribute to the tissue-protective effects of BK through enhanced release of local vasoactive mediators. Besides, streptozotocin diabetes is akin to type 1 (insulin-deficient) diabetes and therefore would not be a suitable model for assessing the metabolic role of BK receptors on the insulin-resistance characteristic of type 2 diabetes.

In summary, the present data confirm and amplify our previous finding that the absence of a functional B2R induces overexpression of the B1R in a variety of tissues. They further confirm previous reports by us and others that the metabolic function of bradykinin, that is, enhanced insulin-dependent glucose transport, is a function of the B2R because its absence causes a state of considerable insulin resistance; they also suggest that this metabolic effect probably is exerted directly, for example, without mediation by local autacoids. Unlike the hemodynamic effects of bradykinin, which, in the absence of B2R can be assumed by the upgraded B1R, the metabolic effects of bradykinin appear to be exerted exclusively through the B1R.

Acknowledgments

This study was supported in part by National Institutes of Health grants R01-HL-58807 and P50-HL-55011.

References

gene in mice eliminates bradykinin action in smooth muscle and neurons.  


Role of the B₂ Receptor of Bradykinin in Insulin Sensitivity
Irena Duka, Sherene Shenouda, Conrado Johns, Ekaterina Kintsurashvili, Irene Gavras and Haralambos Gavras

Hypertension. 2001;38:1355-1360
doi: 10.1161/hy1201.096574

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
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/38/6/1355

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/