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REVIEW

Cardioprotective properties of bradykinin: role of the B_2 receptor

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Following the introduction of angiotensin-converting enzyme (ACE) inhibitors in the treatment of hypertension and ischemic heart disease, there has been increasing interest in the bradykinin-mediated aspects of ACE inhibition. Several preclinical and clinical studies have been conducted using genetically engineered animals or pharmacological agonists and antagonists of the two receptors of bradykinin, B_1R and B_2R. The results have mostly indicated that the B_1R, whose expression is induced by tissue damage, seem to have mostly noxious effects, whereas the constitutively expressed B_2R, when activated, exert mostly beneficial actions. Accumulating evidence in the recent literature suggests that the B_2R have an important role in the process of ischemic post-conditioning that limits the ischemia/reperfusion injury of the myocardium. In this article, we describe a series of experiments conducted on mice submitted to acute myocardial infarct and treated either with ACE inhibition (which produces potentiation of bradykinin resulting in non-selective B_1R and B_2R activation) or with a potent and highly selective B_2R agonist. These data suggest that this latter pharmacological approach offers functional and structural benefits and is therefore a promising cardioprotective therapeutic modality against acute ischemic events.
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INTRODUCTION

The possible role of bradykinin (BK) in cardiovascular regulation has intrigued scientists for many years. A member of the kinin system discovered in the late 1920s, BK is a nonapeptide whose existence was first recognized by Roha e Silva *et al.*[1] from its effects on intestinal smooth muscle. Since then, several other actions of BK were described, including vascular contraction and relaxation, participation in the process of inflammatory reactions, interaction with central and peripheral neural structures, stimulation of synthesis and release of various vasoactive substances, enhanced insulin-dependent glucose transport and utilization, etc.

Circulatory homeostasis is the result of a constant equilibrium between vasoconstrictors (e.g., pressor neurohormonal factors like angiotensin II, catecholamines, vasopressin, endothelin) and vasodilators (like kinins, prostaglandins, NO, etc.). Bradykinin, a tissue hormone that regulates the regional blood flows of vital organs, is an important member of the latter group.[2] The role of BK in cardiovascular regulation under physiological and pathological conditions attracted the interest of clinicians with the advent of angiotensin-converting enzyme (ACE) inhibitors, which in the last two decades have become standard therapy for hypertension, ischemic heart disease and heart failure.[3]

In recent years the work of many investigators has produced a lot of new information regarding the role of kinins in the pathophysiology of hypertension and the prevention of its end-organ complications. Interest in the physiology and pharmacology of BK was prompted by the discoveries that the kininase II, which degrades BK is identical to the ACE;[4] that the BK-potentiating factors isolated from a snake venom could act as antihypertensives in experimental hypertension;[5] that renal prostaglandins synthesized locally were both stimulated by, and found to mediate the action of locally generated kinins;[6,7] that various types of hypertension were associated with, and probably affected by, changes in the renal kallikrein–kinin system;[8] that cyclooxygenase inhibitors, which interrupt prostaglandin synthesis, could induce renal failure through their effects on kinins as well as on the renin–angiotensin system;[9] and, most importantly, by the realization that part of the BP lowering and other effects of ACE inhibitors must be accounted for by potentiation of BK.[10] Notably, the main adverse effects of ACE inhibition, that is, cough and angioedema, have also been attributed to amplified reactions to BK when its enzymatic degradation is impaired.[11]

The cardioprotective effects of ACE inhibition are well recognized, but have been mostly attributed to inhibition of the formation of angiotensin II, whose cardiotoxic (and nephrotoxic) properties were described long ago. The fact that angiotensin AT_1 receptor blockade—which does not involve alteration in BK metabolism—can similarly prevent or minimize end-organ damage has initially focused attention on the angiotensin-mediated actions of both interventions. However, a growing body of evidence in recent years indicates that part of the cardioprotective benefits of ACE inhibition is attributable to the

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diminished degradation and hence accumulation of BK. The following mini-review will highlight some of the studies that show the role of the two BK receptors under conditions of cardiac ischemia and will concentrate on the growing experimental evidence indicating that selective activation of the B_2 type receptor has cardioprotective properties.

BRADYKININ RECEPTORS

A large body of literature over the past several years has explored the physiopharmacology of BK and defined its two receptor types, B_1R and B_2R, which mediate its multiple hemodynamic and metabolic effects.[12] In earlier years this was accomplished mainly via use of peptide analogs with agonistic and antagonistic properties. It has generally been accepted that all the physiologically significant hemodynamic and metabolic actions of bradykinin are exerted via activation of the constitutive B_2R. Indeed, acute and chronic infusion of BK antibodies[13] or selective B_2R antagonists in animals[14,15] and humans[16] was shown to partly reverse the antihypertensive effect of ACE inhibitors; to prevent the cardioprotective action of ACE inhibition in animals submitted to cardiac ischemia/reperfusion injury;[17,18] and to inhibit the amelioration of insulin-dependent glucose transport by ACE inhibitors.[19,20] On the contrary, the B_1R is believed to be mostly unexpressed under normal conditions, but is highly inducible by lipopolysaccharides, bacterial toxins and inflammatory mediators resulting from tissue injury.[21]

More recently, advances in molecular biology permitted the cloning and characterization of these receptors and the creation of genetically engineered mice with deletion of the B_2R[22] or B_1R.[23] Several investigators have used B_2R gene knockout mice to further explore the physiological actions of BK. Compared with their wildtype controls, these animals were shown by some investigators to have higher blood pressure at baseline[24] although others did not confirm this finding.[25,26] However, everybody agrees that inactivation of the B_2R makes animals particularly salt-sensitive, that is, prone to develop earlier and more severe hypertensive response to salt loading.[24–27] They were also found to have less efficient myocardial metabolism with increased propensity to ischemic myocardial injury[28] and increased cardiac oxygen consumption.[29]

There is also evidence that kinins affect preferentially the cardiac and renal perfusion[30] and influence the local redistribution of blood flow within these organs, favoring the subendocardial layers of the myocardium[31] and the papillary region of the kidney,[32] that is, the areas most vulnerable to ischemic tissue damage. It has repeatedly been shown that activation of the endogenenous kallikrein–kinin system by various means can protect the myocardium from ischemia/reperfusion injury. Indeed, the protective effects of ACE inhibitors in this setting can be partially abolished by B_2R antagonists,[17,18,33,34] whereas gene treatment designed to enhance the expression of kinins was shown to attenuate the damage and subsequent remodeling after acute myocardial infarct (AMI).[35]

Another property of the kallikrein–kinin system contributing to its tissue-protective actions is its capacity to enhance the insulin-dependent glucose transport and metabolism,[36,37] which has been shown in both animal and human experiments.[37,38] Indeed, the well-recognized effect of ACE inhibition to improve insulin sensitivity is in part attributed to bradykinin,[39] whereas kininogen-deficient rats were found to be resistant to insulin.[40] This effect of ACE inhibition is also mediated by the B_2R, because it is reversed by selective B_2R antagonists;[19,20] but, unlike the vasodilatory effects of bradykinin, which are exerted via B_2R-mediated activation of the prostaglandin-NO cascade (which can also be stimulated by the B_1R, albeit via different mechanism),[41] this metabolic activity appears to be a direct effect of the B_2R.[20,42,43] Indeed, B_2R gene knockout mice show severe insulin resistance, despite compensatory upregulation of the B_1R.[42]

Cardiovascular complications of hypertension and atherosclerosis are associated with elevation in plasma levels of biochemical markers of inflammation, such as the proinflammatory cytokines interleukin-6 and tumor necrosis factor-α (TNF-α), as well as the resulting stimulation of C-reactive protein.[44–46] High sensitivity C-reactive protein is also significantly correlated with measures of arterial stiffness,[47,48] which may be partly due to angiotensin II[49,50] and partly to metabolic abnormalities, such as diabetes mellitus, with its advanced glycation endproducts, not necessarily correlated to BP. Indeed, ACE inhibition was shown to reverse arterial stiffness in diabetic normotensive patients.[51]

Unlike the B_2Rs, which are constitutively expressed throughout and mediate the majority of the vascular and metabolic actions of kinins, as mentioned above, the B_1Rs are generally absent from normal tissues, but are known to become induced by a variety of pathological conditions, including inflammation, the presence of toxins, cytokines or tissue trauma.[21,52] They were also found to become induced and upregulated by disturbances in circulatory homeostasis, such as induction of experimental hypertension by salt loading, renal artery clipping or exogenous Angiotensin II (Ang II) infusion[41,53] as well as by the normal aging process.[54] But the most important stimulus causing overexpression of the B_1R was found to be the disruption of the B_2R, whether due to functional inactivation via a selective B_2R antagonist or to deletion of B_2R gene.[41,42,55] In such cases, the B_1R can take over some of the vasodilatory functions of the B_1R, but not its metabolic (insulin-sensitizing) function. It was recently reported that in hearts from subjects with end stage cardiac failure, the expression of B_1Rs was significantly increased both at the mRNA and the protein level[56,57] in contrast to the B_2Rs, which were found to be significantly downregulated.[58] A plausible explanation for these findings is that increases in expression and activity of the B_1R may be partly triggered by inflammatory cytokines, such as TNF-α, whose levels increase in chronic heart failure[59–61] and chronic tissue injury, but may also represent a compensatory reaction to diminished functional capacity of the B_2R. At this time it is unclear whether the upregulation of the B_1R under these circumstances may be beneficial by enhancing regional blood flow, thus contributing to tissue protection, or may actually have a detrimental effect by interacting with inflammatory mediators and further exacerbating the inflammatory process.

Nevertheless, the overall experience from basic and experimental studies to date supports the notion that any tissue-protective actions of BK are mostly exerted via activation of the B_2R. Following is a brief review of the data indicating that B_2R activation can protect the myocardium that has been exposed to ischemic injury, and therefore a highly selective B_2R agonist can be cardioprotective, while avoiding the adverse consequences of B_1R activation.

B_2R-MEDIATED CARDIOPROTECTION POST ISCHEMIC INJURY

As mentioned above, the properties of BK under various conditions have been studied extensively with the use of BK analogs with various degrees of agonistic or antagonistic capacity and affinity for the B_1R and B_2R. Earlier studies used mostly agents with BK receptor blocking capacity. One of those, the selective B_2R antagonist HOE-140 or icatibant, has been the agent most widely used in experimental animal and human studies and shown to partly reverse the antihypertensive action of ACE inhibitors[15,16] to prevent the cardioprotective action of ACE inhibition in animals submitted to cardiac ischemia/reperfusion injury[17,18] and to counteract the amelioration of insulin-dependent

glucose transport and utilization that normally occurs with the use of ACE inhibitors.[19,20]

Acute myocardial ischemia causes neurohormonal activation associated with a sharp increase in vasopressor hormones, including the renin system. Ang II was shown long ago to be a cause of acute ischemic myocardial tissue damage leading to microfoci of necrosis and scarring.[62] More recently, we have found that Ang II *in vivo* and *in vitro* stimulates gene overexpression of various vasoactive factors, including the B_1R and B_2R genes[53] as well as a novel gene, the cardiomyopathy-associated 3, also known as myomaxin and recently renamed Xirp2.[63–65] Although this gene's product remains unknown, its contribution to ischemic cardiomyopathy is crucial, as shown by studies in genetically engineered mice.[65] Interestingly, concurrent inhibition of the AT_1 receptor of Ang II with losartan can abolish the BK receptor upregulation.[53] Furthermore, ACE itself can also upregulate the gene expression of both BK receptors by up to 11–22 fold, via a mechanism apparently unrelated to its enzymatic properties.[66] It was reported that in myocardial tissues of rats submitted to acute myocardial infarct there was a significant upregulation of both B_1R and B_2R detectable within 6 h and reaching its peak at 24 h,[57,67,68] with the B_1R expression returning gradually to baseline in the next 3–6 days, whereas the B_2R overexpression appears to last much longer. Thus, myocardial ischemia—whether due to Ang II-induced coronary constriction or to mechanical coronary obstruction—is associated with an upregulation of both BK receptors that would seem to be compensatory, but may also have noxious effects as well.

To further define the function of each BK receptor under ischemic conditions, we conducted a number of experiments in genetically engineered mice with deletion of either the B_2R or the B_1R gene. In B_2R gene knockouts, the B_1R becomes highly upregulated and assumes most of the hemodynamic properties of the B_2R. It also becomes upregulated in response to experimental manipulations inducing hypertension, which do not affect B_2R gene expression.[41] In the absence of B_2R, the B_1R can still contribute to BP lowering by ACE inhibition, as it too can influence vasoactive components of the arachidonic acid cascade, although the effects of the two BK receptors on specific components of the prostaglandin/nitric oxide sequence are different.[27] But, although the B_2R gene knockout mice can still respond to BK with B_1R-mediated vasodilation, they cannot respond to the insulin-sensitizing action of BK. Indeed, using the hyperinsulinemic euglycemic clamp technique, we found that B_2R gene knockout mice become severely insulin-resistant, despite marked B_1R upregulation,[42] evidently because this effect is exerted directly by the B_2R and not via the prostaglandin/NO cascade.

The B_1R gene knockout mice seem to have a normal cardiovascular phenotype at rest, although they have evidence of hypoalgesia and abnormal responses to inflammatory mediators.[23] They also have markedly overexpressed B_2R genes. In a recent series of experiments, we induced AMI by ligation of the left anterior descending coronary artery in B_1R or B_2R gene knockout mice and wild-type controls and evaluated their cardiac function with or without ACE inhibition.[69] Not surprisingly, wild-type mice had a significant decrease in left ventricular (LV) systolic capacity if untreated, whereas if treated with lisinopril they had a significantly lesser loss of LV function. The B_1R gene knockouts, despite overexpression of the B_2R, had a similar degree of decrease in LV systolic function whether treated with ACEI or not. However, the B_2R gene knockouts with overexpressed B_1R, not only had a significantly greater loss of LV systolic capacity than the other groups if left untreated, but also showed a further deterioration, rather than improvement, in LV function if treated with ACEI. These data indicated that potentiation of BK in the absence of B_1R is insufficient to provide full cardioprotection, despite upregulated B_2R; however, in the setting of absent B_2R and upregulated B_1R, the potentiation of BK actually seems to inflict further cardiac tissue damage.[68] In view of the known proinflammatory and noxious B_1R-mediated actions and the loss of the metabolic B_2R-mediated properties that would enhance glucose utilization by the ischemic myocardium, it is not surprising that BK potentiation in the setting of absent B_2R and upregulated B_1R would exert an additional detrimental effect on the injured myocardium.

A growing body of literature in recent years has accumulated data regarding the process of ischemic post-conditioning, which minimizes the myocardial damage following ischemia/reperfusion injury.[70] Although there have been some conflicting data regarding the role of the B_1R in this setting, there is general agreement on the beneficial cardioprotective effects of B_2R activation.[28,71,72] These data provide conclusive evidence indicating that the B_2R have a crucial role in this process of preserving myocardial tissue integrity and functional capacity to various degrees, depending on coexisting conditions and interventions.

In the past it has been theorized that BK receptor agonists, especially those selective for the B_2R, should be considered as means to improve myocardial metabolism in the setting of various cardiovascular disorders, but that the safe therapeutic window between potential beneficial and harmful effects of BK analogs should first be defined.[73] As it is widely accepted that most physiologically important and beneficial effects of BK—including its vasodilatory properties and its insulin-sensitizing effects—are exerted through the constitutively expressed B_2R, whereas the B_1R that is inducible by factors released after tissue injury or inflammation may cause further harm, any BK analog considered for pharmacotherapy should be a BK agonist highly selective for the B_2R. Indeed, a few years back it was reported that coronary artery ligation produced a larger size AMI in kininogen-deficient rats than in normal rats; however, if the former rats were treated for 24 h with infusion of a B_2R agonist, the size of their AMI was the same as that of normal rats, suggesting improved myocardial blood flow in the borders of the ischemic area.[74] By contrast, as mentioned earlier, infusion of a B_2R antagonist has been shown to prevent or reverse the cardioprotective action of ACE inhibition.[17,18]

Treatment with a selective B_1R antagonist in the setting of AMI has no effects of its own; however, it was shown to reverse the cardioprotective action of the angiotensin AT_1 receptor blocker irbesartan[75] despite the fact that BK potentiation does not contribute to the vasodilatory/antihypertensive actions of AT_1 receptor blockers. This finding was attributed to the cross-talk between AT_1 and B_1R genes. Indeed, there is emerging evidence that in addition to the widely investigated hemodynamic and metabolic actions of the ACE, Ang II and BK, they also have transcriptional regulatory properties and interactions that have not yet been fully appreciated: Ang II stimulates gene expression of the BK receptors, an action inhibited by the AT_1 receptor blocker losartan,[53] and ACE inhibitors have been reported to affect gene expression of BK receptors in the absence of ACE,[76] whereas ACE itself can enter the nucleus of cells and stimulate gene expression of BK receptors.[66,77] Incidentally, there is a large body of literature exploring the molecular signaling interactions elicited by vasoactive factors and their possible implications on carcinogenesis.[78] Such interactions further complicate the interpretation of results from pharmacological interventions altering the function of these vasoactive factors and the molecular mechanisms mediating these results. Nevertheless, the hemodynamic and histological results are indisputable, even when the underlying molecular mechanisms are unclear.

Recently Dr Gobeil and colleagues from Sherbrooke University, Canada, came up with a series of BK peptide analogs, of which one, the compound NG 291 (Hyp^3, Thi^5 ^{N}Chg^7 Thi^8-BK) was a B_2R agonist with the highest potency, selectivity and affinity for the B_2R and the highest resistance to proteolytic enzymes.[79] Using this agent, we conducted a series of experiments designed to evaluate the capacity of selective B_2R activation to minimize ischemic myocardial damage. After inducing AMI via ligation of the left anterior descending coronary artery in mice, we initiated a continuous infusion of this agent for 1 week via osmotic minipump implanted subcutaneously in their scapular area. We then compared the functional LV capacity and the extent of cardiac tissue damage and remodeling, as well as the gene expression pattern of a number of factors related to myocardial tissue inflammation, perfusion and metabolism, between actively treated mice and control mice receiving the vehicle saline solution. We found that actively treated mice had a significantly attenuated decrease in systolic function compared with saline-treated mice, as shown by the better ejection fraction and better fractional shortening, as well as a lesser degree of cardiac remodeling and a closer to normal body weight to heart weight ratio. Despite the known vasodilatory effects of the B_2R, systolic blood pressure at end-point was normal in the B_2R agonist-treated mice, whereas it tended to be depressed in the saline-treated controls, evidently reflecting the diminished cardiac systolic capacity and decreased cardiac output. The infarct size tended to be smaller, though not significantly so, in actively treated mice, but there was a significant increase in markers of myocardial cell necrosis and apoptosis in the saline-treated group.[80]

Analysis of expression patterns of selected genes to factors related to tissue injury, inflammation and metabolism, was performed in cardiac tissues from the two MI groups, in comparison with cardiac tissues of sham-operated mice with or without B_2R agonist treatment. The results indicated that acute myocardial ischemia induced significant upregulation of these genes (that is, the B_1R, B_2R, eNOS, TNF-α, PDk4 and cardiomyopathy-associated 3/Xirp2), whereas the B_2R agonist itself produced no difference in pattern of gene expression in the myocardium of sham-operated mice, whose patterns were similar to the saline-treated sham-operated ones. However, the active treatment produced a profound attenuation of the upregulation of these genes in the actively treated MI mice to levels close to those of the sham-operated normal animals. As these factors are reported to regulate tissue perfusion, metabolism and apoptosis, we concluded that their tendency to normalization must reflect the beneficial influence of the experimental treatment.

These experiments prove that a continuous infusion for 1 week of a potent and highly selective B_2R agonist of bradykinin initiated at the time of occurrence of an acute MI can diminish the extent of myocardial damage. This is shown by the significantly improved indices of left ventricular function and cardiac tissue remodeling, as well as the significantly attenuated pattern of inflammation-related tissue gene expression, which explains the structural and functional benefits of the treatment. With this approach, it is possible to take advantage of the beneficial effects of B_2R activation, while avoiding the nefarious consequences of upregulation and stimulation of the B_1R. In fact, this approach could also be utilized in other conditions of myocardial ischemia, for example, surgical procedures, where preservation of myocardial tissue may be enhanced by optimizing glucose metabolism. One intervention that has recently been re-introduced to this aim is the 'GIK' treatment, that is, a controlled glucose–insulin–potassium infusion[81] that was shown to benefit diabetics with coronary artery disease undergoing cardiac surgery. A B_2R agonist infused along with the GIK components would further enhance the benefits of this intervention by promoting optimal glucose utilization by cardiomyocytes, something particularly useful for patients with type II diabetes mellitus, who are already at higher risk of ischemic cardiac events and adverse long-term consequences of such events.

CONCLUSIONS

In this review, we describe the results of a recently conducted series of experiments on wild-type or genetically engineered mice with deleted B_1R or B_2R, submitted to acute MI and treated with an ACE inhibitor or with a highly selective B_2R agonist bradykinin analog. Overall, the results of these studies offer additional support to the notion that unselective potentiation of bradykinin may lead to mixed results, as activation of the B_1R appears to have predominantly noxious effects, whereas selective activation of the B_2R has predominantly beneficial effects in terms of both functional and structural cardioprotective actions. We therefore conclude that treatment with a potent and highly selective B_2R agonist, initiated immediately after the occurrence of an acute ischemic event, should be further explored as a potential therapeutic option in these circumstances; indeed the data so far offer 'proof of concept' for the value of this pharmacological approach, but further studies are needed to ascertain the safety and long-term efficacy of this intervention.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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