

Role of the bradykinin B₂ receptor in the maturation of blood pressure phenotype: lesson from transgenic and knockout mice

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Abstract

The binding of bradykinin (BK) to its B₂ receptor results in a wide spectrum of biological effects including vasodilation, smooth muscle contraction and relaxation, pain, and inflammation. In order to gain a better insight into the physiological function of this potent vasoactive peptide, murine models have been created by the use of gene insertion or deletion. The results of studies using these strategies are revisited in the present article. In transgenic mice harboring the human BK B₂ receptor cDNA (chBKR), expression of the transgene was identified in the aorta, brain, heart, lung, liver, kidney, uterus and prostate gland by RT-PCR Southern blot analysis. These mice displayed an exaggerated hypotensive response to intra-aortic injection of BK, whereas the blood pressure of knockout mice, homozygous for targeted disruption of the endogenous gene, was insensitive to BK. Two transgenic mouse lines expressing the human BK B₂ receptor showed a significant reduction of systolic tail-cuff blood pressure (84 ± 1 mm Hg, $n = 28$; 80 ± 1 mm Hg, $n = 24$; $P < 0.001$) compared with the control littermates (97 ± 1 mm Hg, $n = 52$). Systolic blood pressure was elevated in BK B₂ receptor knockout mice (124 ± 1 mm Hg, $n = 38$). In heterozygous mice, systolic blood pressure was similar to that of controls until 5 month-old, then it raised to the elevated levels of knockout mice at 7 months of age. Together these data indicate that kinins acting through the B₂ receptor play a role in the development of the blood pressure phenotype. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Gene; Transgenic animals; Kinins; Receptors; Blood pressure

1. Introduction

Bradykinin (BK) exerts a wide spectrum of biological functions including vasodilation, pain and inflammation. At the present, two receptor subtypes for kinins are known: the B₁ subtype, characterized by a higher affinity for des-Arg⁹ BK, and the B₂ subtype, the most important receptor for BK under

Abbreviations: BK, Bradykinin; chBKR, Human Bradykinin B₂ Receptor cDNA; RSV, Rous sarcoma Virus

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normal conditions (Rhaleb et al., 1991). Circumstantial evidence based on the use of potent and selective receptor antagonists indicates that BK, acting on the B₂ receptor, could play a role in the regulation of blood pressure (Madeddu et al., 1995). Recent advances in molecular biology has made it possible to produce permanent changes in the gene encoding for the BK receptor. Disruption of the BK B₂ receptor gene has been obtained in mice by the methodology of gene targeting via homologous recombination (Borkowski et al., 1995). More recently, transgenic mice harboring the human BK B₂ receptor transgene (RSV-CHBKR) under the control of the Rous sarcoma virus 3'-LTR promoter have been created (Wang et al., 1997). In the present article, we review the results of recent studies in which the above mentioned murine models were used to ascertain the role of BK in the regulation of blood pressure.

2. Material and methods

Knockout mice were provided by Merck Research Laboratory (Rahway, NJ). Gene targeting was performed by transfecting embryonic stem cells derived from 129Sv/Ev mice with a vector designed to disrupt the entire coding sequence for the B₂ receptor by homologous recombination (Borkowski et al., 1995). Injection of 129Sv/Ev embryonic cells, carrying the targeted mutation, into C57Bl/6J blastocysts, produced chimeric mice. They were mated with 129Sv mice and only the offspring that were heterozygous for the knockout (thus having both sets of chromosomes of 129Sv origin) were used for subsequent mating to homozygosity. In homozygous mice, disruption of the B₂ receptor gene was confirmed by genetic, biochemical, and pharmacological analyses. The knockout mice were compared with 129Sv/J and with heterozygous mice obtained by breeding pairs of homozygous mice and wild type controls.

Transgenic mice harboring the human BK B₂ receptor cDNA under the control of the Rous sarcoma virus 3'-LTR promoter were created by Chao et al. (Wang et al., 1997). Expression of the transgene was identified in the aorta, brain, heart, lung, liver, kidney, uterus and prostate gland by RT-PCR Southern blot analysis.

Mice were housed at constant room temperature ($24 \pm 1^\circ\text{C}$) and humidity (60%) with a 12-h light/dark cycle. All procedures complied with the standards for the care and use of animal subjects as stated in the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, National Academy of Sciences, Bethesda, MD).

Systolic blood pressure during development was measured by the tail-cuff plethysmography method in unanesthetized male mice. Tail-cuff systolic blood pressure is defined as the inflation pressure at which the pulse waveform becomes indistinguishable from baseline noise. Calibration was performed with a mercury sphygmomanometer.

The mean blood pressure response to intra-aortic bolus injection of BK (3 nmol/100 g body weight) was evaluated in conscious male mice. Blood pressure was measured by the use of an indwelling femoral catheter connected to a Statham transducer.

All data are expressed as mean \pm SEM. Multivariate-measures ANOVA was used to test for interaction between time and grouping factor. Univariate ANOVA was then used among groups and over time. A *P*-value less than 0.05 was considered as statistically significant.

3. Results and discussion

Clinical studies have shown that tissue kallikrein levels are reduced in essential hypertension (Zinner et al., 1978). Conversely, a dominant allele expressed as high urinary kallikrein excretion may be associated with a decreased risk of essential hypertension (Berry et al., 1989). In addition, a restriction length fragment polymorphism of the rat tissue kallikrein gene cosegregates with elevated blood pressure levels (Pravanec et al., 1991). While these studies indicate a correlation between kallikrein and blood pressure, to our knowledge there is no information regarding variants of the B₂ receptor gene linked to hypertension. Unfortunately, linkage studies of complex diseases — such hypertension — have limited power to detect genes of modest effect. Inconsistency of studies performed in different populations appears to occur very frequently. For in-

stance, the candidate gene encoding for angiotensin converting enzyme was found to be associated to hypertension in stroke-prone spontaneously hypertensive rats by two different groups (Hilbert et al., 1991; Jacob et al., 1991), but was found not to cosegregate with blood pressure in another study (Kreutz et al., 1995). Among possible explanations of this discrepancy, one important point is that the blood pressure phenotype is usually examined at fixed ages. This approach might preclude the observation of correlation if the gene penetrance is modest and/or long-term exposure to favoring environmental factors is needed for phenotype expression. Therefore, developmental analysis could provide the critical tool to unmask the correlations. Given the length of the human life-span, developmental analysis is more feasible to be performed in animal models.

Another limitation of segregation approach is that failure to find a linkage between hypertension and markers of candidate gene mutation is not conclusive to exclude association between blood pressure and the relevant gene. Conversely, a difficult problem remains even when a candidate gene has been identified by segregation. In fact, correlation does not necessarily imply a proof of causation.

These drawbacks led geneticists to use gene manipulation approach aimed to synthesize the phenotype of interest either by gene targeting (to disrupt existing information) or by adding a suitable transgene (to enhance gene expression and the levels of gene product). The lack of function obtained by gene knockout is informative in assigning primary causation, but rarely occurs in essential hypertension. Indeed, only in a small percentage of hypertensive patients, the disease state is due to defects in single genes inherited as simple recessive Mendelian factors (Lifton, 1996). In the majority of cases, essential hypertension is the result of combinations of genetic variants less drastic than the absence of gene function. This implies that: (1) individually, each genetic variation may not be sufficient to cause deviation from normality, and (2) the disease phenotype of null mutants might actually overestimate the contribution of the candidate gene to hypertension. As indicated in Table 1, the power and accuracy of the synthetic approach can be enhanced in murine models by evaluating the developmental changes of the pheno-

Table 1

How the power of a synthetic approach can be augmented

By introducing developmental analysis of the phenotype
 By titrating gene dosage-effects
 (from null mutation to multiple copies of the relevant gene)
 By evaluating gene expression in animals with different
 genetic backgrounds

type and by titrating gene dosage-effects (from null mutation to multiple copies of the relevant gene).

Fig. 1 cumulates data from two separate studies performed by our group in mice with disruption of the BK B₂ receptor gene (Madeddu et al., 1997) and by Wang et al. in transgenic mice expressing the human B₂ receptor gene (Wang et al., 1997). Wild type animals were also included as a reference. In addition, heterozygous mice carrying only one copy of the wild-type gene were studied. As shown in the left panel of Fig. 1, intra-arterial injection of BK did not produce any blood pressure change in the knockout mice, while in transgenic animals the blood pressure fall was exaggerated as compared to wild-type controls. The responses to exogenous BK were inversely correlated with basal blood pressure levels in the four groups examined, with transgenic mice being hypotensive and the knockout mice hypertensive as compared to wild-type controls (Fig. 1, right panel).

Heterozygous animals carrying one copy of the gene are important not only because they help understand the effect of varying the number of functional copies of the targeted gene but also because they resemble more closely a condition of “partial deficiency” that might occur in human hypertensive patients. The vasodepressor response to BK was not altered in these heterozygous animals, a finding that is compatible with a great deal of redundancy or spare capacity of the B₂ receptor (i.e., only a fraction has to be occupied to elicit full response). Consistently, the blood pressure of these animals was not altered at 5 months of age as compared with wild-type controls. However, the importance of examining the phenotype from early developmental phases to complete maturation is outlined by the finding that a progressive blood pressure elevation was observed in heterozygous animals with aging (Fig. 2). Thus, interactions between the gene encod-

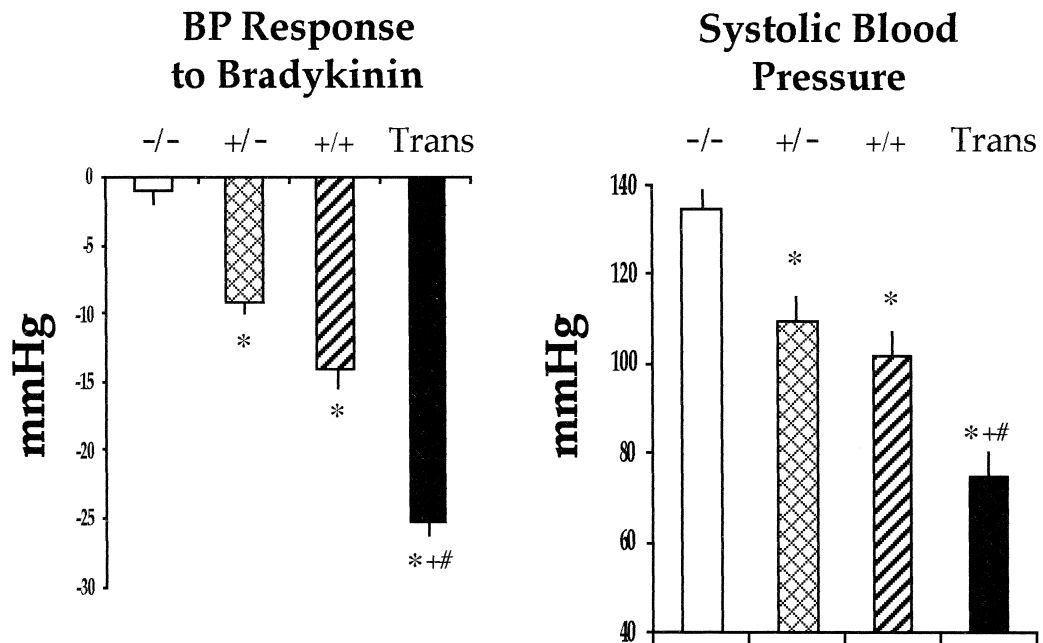


Fig. 1. Left panel shows the absolute mean blood pressure changes induced by an intra-aortic bolus injection of bradykinin (3 nmol/100 g body weight). Right panel shows the systolic blood pressure levels detected at 5 months of age under basal conditions. Only male mice were studied: bradykinin B₂ receptor knockouts (-/-, *n* = 10), heterozygous (+/-, *n* = 10), wild type controls (+/+, *n* = 10), and transgenic mice carrying the human bradykinin B₂ receptor gene (Trans, *n* = 24). Values are mean ± SEM. **P* < 0.05 vs. knockouts, +*P* < 0.05 vs. heterozygous, #*P* < 0.05 vs. wild type mice.

ing for the BK B₂ receptor and other genes or environmental factors might be developmentally regulated.

Although neither the transgenic nor the knockout mice showed alterations in the expression pattern of renin and ACE genes (Madeddu et al., 1997; Wang et al., 1997), an enhanced sensitivity to exogenous or endogenous angiotensin II has been demonstrated in the knockouts (Madeddu et al., 1997, 1998). Interestingly enough, the null mutation (i.e., the lack of the BK B₂ receptor gene) yields a similar hypertensive response to salt loading in two different colonies (Alfie et al., 1996; Madeddu et al., 1997). However, our study showed elevated blood pressure levels also under normosmotic conditions and pressure sensitivity to exogenous angiotensin II, whereas this was not the case in Alfie's study. Although the two colonies were originated from the same source (Merck Research Laboratory) and although genetic analysis has confirmed the disruption of the gene, it is still possible that unknown mutations have occurred in the

genetic background either in stocks provided by Merck or in colonies established later in the two laboratories. Searching for differences in genetic background among substrains could be very informa-

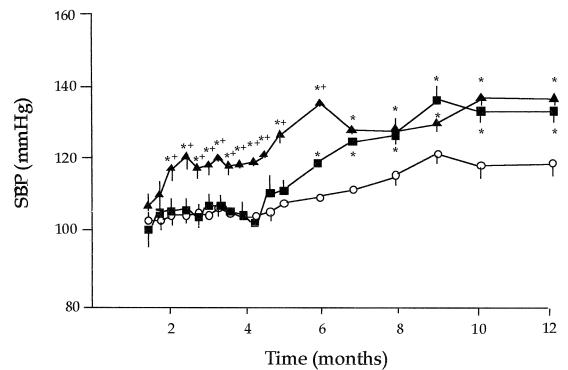


Fig. 2. Age-related changes in systolic blood pressure (SBP) in bradykinin B₂ receptor knockouts (triangles, *n* = 38), heterozygous (squares, *n* = 12), and wild type controls (circles, *n* = 33). Values are mean ± SEM. **P* < 0.05 vs. wild type mice, +*P* < 0.05 vs. heterozygous mice.

tive to understand gene interactions, but it also represents a daunting task.

In conclusion, results obtained from studies on transgenic and knockout animals support the hypothesis that the binding of BK to the B₂ receptor is important for the maintenance of blood pressure homeostasis and encourages the search for functional variants of the receptor in hypertensive patients.

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