Letters to the Editor

Effect of Chronic Blockade of the Kallikrein-Kinin System on the Development of Hypertension in Rats

To the Editor:

We found some incorrect statements in an article prepared by Nour-Eddine Rhaleb et al titled “Effect of Chronic Blockade of the Kallikrein-Kinin System on the Development of Hypertension in Rats,”1,3 and we consider it our duty to point out the errors (and other related irregularities) and to provide the correct information for the readers of Hypertension. It should be mentioned at the outset that Dr Majima was invited to Dr Rhaleb’s laboratory by the director of that laboratory, Dr Oscar A. Carretero, to conduct the experiments in question.

In the Discussion section (pp.126, right column, ll. 28 to 38), appears the following statement’: “(1) normal BN [Brown Norway] rats were given a subcutaneous infusion of Ang II or high salt diet alone or combined with icatibant (given intraperitoneally) and (2) BNK [Brown Norway Katholiek] rats were given a subcutaneous infusion of a nonpressor dose of Ang II or high salt diet...we were unable to reproduce any of their findings or those of Madedu et al.” However, the only 2 experiments that Majima performed with Dr Rhaleb, during a stay of nearly 1.5 months, focused solely on the effects of high salt diet in normal BN rats and on those of the infusion of Ang II in kininogen-deficient BNK rats and normal BN rats. Majima’s work with Dr Rhaleb (as far as it is possible to judge) confirmed our original results. Accordingly, the statement “we were unable to reproduce any of their findings” is not correct, and we can find no justification for it.

In addition, there is no explanation in the text of why the mean blood pressure during week 1 was lower (by ~7 mm Hg) than that in the pretreatment period. According to our data gathered over a period of ~10 years, the systolic blood pressure of mutant kininogen-deficient BNK rats (130 ± 2 mm Hg) is not different from that of normal BN Kitasato rats (127 ± 2 mm Hg), and the blood pressure of these rats showed no essential change during vehicle infusion (as originally published in Hypertension, Table 1).2

According to the “Instructions to Authors” of the recent issue of Hypertension, for any acknowledgments, a statement “signed by the corresponding author, stating that those acknowledged in the manuscript have seen and approve of the manner in which their names are mentioned” is required. However, Majima was not asked for permission to include his name in the Acknowledgments section nor was he given an opportunity to see the manuscript before its submission (or acceptance) for publication. His repeated requests to the editor-in-chief to be sent a copy of the statement sent by the authors to the editor-in-chief have not generated any response whatsoever.

Masataka Majima
Professor of Pharmacology
Kitasato University School of Medicine

Makoto Katori
Emeritus Professor of Pharmacology
Kitasato University School of Medicine


Role of Kinins in Blood Pressure Regulation: Reality or Fiction

To the Editor:

We read with interest the article entitled “Effect of Chronic Blockade of the Kallikrein-Kinin System on the Development of Hypertension in Rats” by Rhaleb et al.1 By a pharmacological approach, the authors evaluated if endogenous kinins can mitigate hypertension induced by Ang II or mineralocorticoids. Although unable to confirm the hypothesis under challenge, the authors showed that icatibant prevents the early antihypertensive effect induced by ramipril in uninephrectomized deoxycorticosterone acetate (DOCA)-salt treated rats. Thus, at least under ACE-inhibition, kinins may become important in contrasting mineralocorticoid-induced hypertension.

Recent availability of selective kinin antagonists and genetically modified animals lacking or overexpressing components of the kallikrein-kinin system (KKS) has renewed the interest in the area. However, enthusiasm generated by positive reports2–3 has been criticized by Rhaleb et al.1 unable to confirm the higher BP of kinin B2 knockout mice (B2-KO) and the enhanced pressor response to Ang II or mineralocorticoids in animals with interrupted kinin receptor signaling. Here, we report our concern regarding the sensitivity of these negative studies. In experiments aimed to challenge Ang II action under icatibant, Rhaleb et al used groups of 4 to 5 animals. Considering the small sample size and the large over-time BP variability in controls (20 mm Hg), it can be calculated that the power of the test barely reaches 0.28. Consequently, because of the low sensitivity of measurements, only astonishing BP changes caused by kinin B2 blockade would be significant. Under these conditions, a negative result may configure a type 1 error. Rhaleb et al also refer to a previous work of theirs, affirming that B2-KO are not sensitive to mineralocorticoids.4 The authors showed that BP was increased by 27 mm Hg in DOCA-treated B2KO versus 16 mm Hg in controls (P=0.07). They conclude that the absence of the receptor does not make the difference. However, we argue that with such a level of probability, no strong conclusion can actually be reached. Furthermore, Rhaleb has superimposed high salt and uninephrectomy to DOCA-treatment. This is at variance with our approach, because we preferred to avoid any maneuver that could suppress kallikrein and mask the importance of the system in mineralocorticoid-induced hypertension.5 Thus, we regret to say that Rhaleb’s methodology differs from ours and that his reference to our article is partial and incorrect.

Quite singular is Rhaleb’s tendency of unequally emphasizing the points of agreement and disagreement in the field. The findings from either laboratory are consistent regarding the issue that acute or chronic administration of kinin antagonists does not alter the cardiovascular phenotype of rodents under conditions of salt loading. Furthermore, agreement exists regarding sodium-sensitivity of B2-KO, although our strain was more prone to the toxic effect of high salt.2–8 This prompted us to formulate the hypothesis that genetic drift was causing phenotypic changes in small-size colonies present at various laboratories.9

The variability of B2-KO cardiovascular phenotype seems to confirm our opinion. At variance with Rhaleb’s data, Gavras’ group8 and ourselves5 documented that B2-KO have higher BP and display an enhanced compensatory statement of inducible B receptor. Eventually, B2-KO develop heart remodeling, myocardial damage, and failure with aging.1 Sensitivity of B2-KO to Ang II or renin-dependent hypertension was documented by us and...
Cervenka\(^6\) but negated by others.\(^1\) The theory of genetic drift driving B\(_2\)-KO toward fixation of divergent phenotypes was reinforced by the observation that one mutant line developed testicular throratoma at our laboratory (Madeddu P, unpublished observations), a feature typical of some J129-substrains. The absence of the above pathology in other related lines of B\(_2\)-KO favors the possibility of a nonhomogeneous genetic background among mutants. In line with this theory, Dr Milia, an investigator of our team visiting Max Delbruck Center (Berlin, Germany), was not able to find any alteration in the cardiovascular phenotype of B\(_2\)-KO (J129 genetic background) derived from a breeding pair that I personally supplied to that institution.\(^10\) In our laboratory, we have confirmed these negative results on the progeny of the German stock kindly returned to us by Prof Luft. Interestingly enough, during the same set of measurements, B\(_2\)-KO mice of a different background (C57/BL6) showed elevated BP and HR (118±3 mm Hg and 628±12 bpm versus 100±3 mm Hg and 531±17 bpm in wild type, \(P<0.01\)) and ventricular hypertrophy (Madeddu et al, unpublished observations). Our observation that the hypertensive, hypertrophic phenotype of B\(_2\)-KO is reproduced in a different genetic background favors a role of B\(_2\) receptor signaling in the regulation of cardiovascular function. Altogether these results indicate that (1) given the redundancy of mechanisms controlling BP, the contribution of kinins may be detected only using high-power experimental strategies; and (2) the lack of phenotypic homogeneity of B\(_2\)-KO may be attributable to random fixation of alleles caused by genetic drift. Microarray analysis could be extremely useful in elucidating the role of otherwise unappreciated mechanisms responsible for the variability among related substrains.\(^10\) Out of these reasons, we hope that apparently conflicting results and queries will not discourage more in depth research in the field of KKS.

Paolo Madeddu  
Gene Therapy Section  
INBB National Laboratory  
Osilo, Italy


Response

We have shown that kinins play a role in the antihypertensive effect of ACE inhibitors in mineralocorticoid hypertension in mice\(^1\) and rats,\(^2\) but only during the early phase of treatment (1 week); they do not participate in the long-term antihypertensive effect of ACE inhibition (4 to 6 weeks) in hypertension induced by mineralocorticoids in mice\(^1\) or by aortic coarctation in mice\(^1\) and rats.\(^3\)

In our recent study, we found that chronic blockade of B\(_2\) kinin receptors did not enhance the effect of chronic infusion of a subpressor dose of angiotensin II,\(^2\) as shown by 2 separate experimental protocols. In one of these protocols, involving 5 to 7 rats per group, variation was minimal, while in the other we only observed marked variation at 2 points by the end of drug infusion.\(^2\) There was no tendency toward an enhanced pressor response to angiotensin II in rats chronically infused with a B\(_2\) kinin receptor antagonist.

Madeddu suggests that uninephrectomy could suppress kallikrein activity and mask the important role of the kallikrein-kinin system in mineralocorticoid hypertension, which is not supported by the findings of other authors.\(^4\)\(^5\) Indeed, those authors found that kallikrein activity more than doubled in rats that were uninephrectomized and given high salt plus deoxycorticosterone (DOCA-salt). Therefore, we took those findings into account when we designed our study on the role of kinins in DOCA-salt hypertension.

Madeddu used data recently published by Gavras’s group as a confirmation of his previous finding that kinin B\(_2\) knockout (B\(_2\)-KO) mice are hypertensive.\(^6\)\(^7\) However, the blood pressures (BP) reported by Gavras’s group were only slightly higher and in some cases no different between B\(_2\)-KO and control mice.\(^6\) In addition, unlike Madeddu’s study,\(^8\) 2-kidney, 1-clip hypertension resulted in a similar increase in BP and cardiac hypertrophy in both strains. Madeddu’s group reported earlier that B\(_2\)-KO mice at \(\approx\)4 months of age were hypertensive compared with wild-type controls,\(^5\)\(^6\) findings that others, including our laboratory, could not confirm.\(^1\)\(^10\)\(^11\) Recently, Milia et al compared blood pressure of B\(_2\)-KO mice that originated from Madeddu’s laboratory to controls (129Sv/J).\(^11\) They used the telemetry method, a state-of-the-art method for BP determination in mice, to assess BP and heart rate; yet, they were not able to confirm hypertension or fast heart rate in B\(_2\)-KO mice. Therefore, the “genetic drift” hypothesis postulated by Madeddu may not apply.\(^12\)

Moreover, we recently compared B\(_2\)-KO mice to 129X1/SvJ (a control commonly used by Madeddu’s group) and 129/SvEvTac mice (a control used by our laboratory) at 1, 2, 3, and 6 months of age but found no significant difference in tail-cuff systolic pressure or mean blood pressure (MAP) measured directly in awake mice.\(^13\) Direct MAP measurement significantly correlates with BP recorded simultaneously via a telemetric device (\(r^2=0.97; P<0.0001\)) (N.E. Rhaleb and O.A. Carretero, personal observation, 2001). Others have measured MAP in awake mice but found that abolition of B\(_2\) receptors does not cause adult hypertension under normal conditions.\(^10\) In response to a previous Letter to the Editor in Hypertension by Madeddu’s group,\(^12\) we invited him to exchange B\(_2\)-KO mice with our laboratory and see whether their mice have different phenotypes than ours. However, conveniently Madeddu’s colony is no longer available. On the other hand, our B\(_2\)-KO colony is still available for any laboratory, including Madeddu’s, that is interested in assessing the role of kinins in the cardiovascular system.

Nour-Eddine Rhaleb  
Xiao-Ping Yang  
Oscar A. Carretero


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Masataka Majima and Makoto Katori

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