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,” \(^1\) \(^2\) 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 \(\text{Hypertension}\). 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 Mageddu et al.\(^3\)\(^4\) 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 \(\sim 7\) mm Hg) than that in the pretreatment period. According to our data gathered over a period of \(\geq 10\) years, the systolic blood pressure of mutant kininogen-deficient BNK rats (130 \(\pm\) 2 mm Hg) is not different from that of normal BN Kitasato rats (127 \(\pm\) 2 mm Hg), and the blood pressure of these rats showed no essential change during vehicle infusion (as originally published in \(\text{Hypertension}\), Table 1).\(^5\)

According to the “Instructions to Authors” of the recent issue of \(\text{Hypertension}\), 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.

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1. Rhaleb NE, Yang XP, Nanba M, Shesely EG, Carretero OA. Effect of chronic blockade of the kallikrein-kinin system on the development of hypertension in rats. \(\text{Hypertension}\).
2. Rhaleb NE, Yang XP, Nanba M, Shesely EG, Carretero OA. Effect of chronic blockade of the kallikrein-kinin system on the development of hypertension in rats. \(\text{Hypertension}\).
4. Rhaleb NE, Yang XP, Nanba M, Shesely EG, Carretero OA. Effect of chronic blockade of the kallikrein-kinin system on the development of hypertension in rats. \(\text{Hypertension}\).
5. Rhaleb NE, Yang XP, Nanba M, Shesely EG, Carretero OA. Effect of chronic blockade of the kallikrein-kinin system on the development of hypertension in rats. \(\text{Hypertension}\).
Cervenka⁹ but negated by others.¹ The theory of genetic drift driving B₂-KO toward fixation of divergent phenotypes was reinforced by the observation that one mutant line developed testicular thromatosis 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 B₂-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 B₂-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, B₂-KO mice of a different background (C57/L6) 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 B₂-KO is reproduced in a different genetic background favors a role of B₂ 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 B₂-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.

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Response

We have shown that kinins play a role in the antihypertensive effect of ACE inhibitors in mineralocorticoid hypertension in mice¹ 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 mice³ or by aortic coarctation in mice¹ and rats.³

In our recent study, we found that chronic blockade of B₂ 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 B₂ 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 B₂ knockout (B₂-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 B₂-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 B₂-KO mice at ≥4 months of age were hypertensive compared with wild-type controls,⁶,⁹ findings that others, including our laboratory, could not confirm.¹⁰,¹¹ Recently, Milia et al compared blood pressure of B₂-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 B₂-KO mice. Therefore, the “genetic drift” hypothesis postulated by Madeddu may not apply.¹² Moreover, we recently compared B₂-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 (r²=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 ablation of B₂ 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,¹¹ we invited him to exchange B₂-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 B₂-KO colony is still available for any laboratory, including Madeddu’s, that is interested in assessing the role of kinins in the cardiovascular system.

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