Letters to the Editor

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Chronic Salt Loading and the Expression of Adenosine Receptor Subtypes

To the Editor:

We read with great interest the paper by Zou et al[1] entitled "Effect on Chronic Salt Loading on Adenosine Metabolism and Receptor Expression in Renal Cortex and Medulla in Rats" in the January 1999 issue of *Hypertension* regarding the effect of chronic salt loading on adenosine metabolism and receptor expression in renal cortex and medulla in rats. It was reported in this article that chronic salt loading influences the expression of adenosine receptor subtypes (A_1, A_{2A}, A_{2B}, and A_3) in the renal cortex and medulla. The authors report that an immunoreactive band of 39 kDa was identified when the membranes were probed with anti-A1 receptor antibody. Further, bands of 45 ($A_{2A}R$), 50 ($A_{2B}R$), and 52 kDa (A_3R) were detected when membranes were probed with anti-A_{2A}, A_{2B}, and A_3 receptor antibodies, respectively.

We believe that the molecular masses of $A_{2B}R$ and A_3R are totally incorrect and that the molecular mass of A_1R is also questionable. It has been reported[2–6] that the molecular weights of A_{2B}, A_3, and A_1 receptor proteins are in the range of 36 to 37 kDa compared with 50, 52, and 39 kDa, respectively, as reported in the Zou et al[1] paper. Recently, in another study,[6] the molecular weight of the A_{2B} adenosine receptor is being reported to be different for the same antibody, which was obtained from Alpha Diagnostics. This is quite disturbing and needs to be investigated by scientists in the field, which are using these antibodies. We are currently analyzing these antibodies for their specificity and cross-reactivity using recombinant receptors expressing only single receptor proteins. As far as we know, no investigator has ever characterized these antibodies. Unless we have the data to support that these antibodies are specific in nature, one has to be very careful in reaching this conclusions since all the adenosine receptors except A_{2A} have the same molecular masses. Therefore, in our opinion, it is possible that some non-specific protein bands reported in the above study[1] might have been detected by these investigators[1] in the range of 50 to 52 kDa due to the polyclonal nature of antibodies. These bands have been misunderstood by the authors as A_{2B}, A_3, and A_1 proteins at 50, 52, and 39 kDa sizes instead of 36 to 37 kDa.

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Response

All antibodies used in our studies were purchased from Alpha Diagnostics, Inc, which were purified by affinity chromatography.[1] When the membranes carrying renal cortical and medullary tissue proteins were probed with these antibodies, only one single dark immunoreactive band for each receptor was detected in the whole membrane with a few exceptions in the renal medulla, although we only presented a small part of each gel document in order to incorporate 4 gels into one figure. The molecular sizes of detected receptors were consistent with those indicated by the manufacturer. The specificity of these antibodies was confirmed by competing with excess corresponding peptides and recombinant adenosine receptor proteins. We believe that the immunoreactive bands recognized by these antibodies represent corresponding adenosine receptors.

Nayeem et al questioned the molecular masses of detected adenosine receptors in our studies based on the predicted molecular sizes of deduced amino acids from receptor cDNA sequences, as indicated in those review articles cited by them. In fact, the predicted molecular size of proteins deduced from cDNA sequence does not necessarily represent the actual molecular size of detected or purified proteins from a variety of tissues. Since the splicing of RNA during transcription may produce different sizes of mRNA in different tissues or different species and since the posttranslational processing or membrane assembly may alter the sizes of translated receptor proteins, it is not surprising that actually detected receptor proteins by Western blot analysis can exhibit different molecular sizes in different tissues or species. With respect to adenosine receptors, only a few studies using Western blot analyses (including our work published in *Hypertension*[1]) have been done to detect their expression. The results show that the molecular sizes of detected adenosine receptors in different tissues are inconsistent and different from the predicted sizes.[1–4] In their own work, Marala and Mustafa,[4] the colleagues of Nayeem, have also reported that the size of the detected A_{2A} receptor by Western blotting was different from the predicted size of this receptor.[5] It is possible that the heterogeneity of adenosine receptor expression, posttranslational processing, or membrane assembly may occur in different tissues or species. The heterogeneity or differences between predicted molecular size from deduced amino acids and purified protein are also found in other proteins. For instance, hypoxia-inducible factor-1α (HIF-1α), a transcription factor that we have been working with, has a predicted size of 93 kD by deduced amino acids, but purified or immunoreactive HIF-1α protein is 120 kD.[6,7] Therefore, the predicted molecular sizes of 36 to 37 kD based on A_{2B}, A_3, and A_1 receptor cDNAs as cited by Nayeem et al do not necessarily represent the sizes of purified or immunoreactive receptor proteins, and we have confidence in the results that we have reported.

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