Author's response to reviews

Title: Disruption of the endothelin A receptor in the nephron causes mild fluid volume expansion

Authors:

Deborah Stuart (Deborah.stuart@hsc.utah.edu)
Sara Rees (sara.rees@has.utah.edu)
Stephanie K Woodward (stephanie.k.woodward@gmail.com)
Robert Koesters (robert.koesters@upmc.fr)
Kevin A Strait (kevin.strait@hsc.utah.edu)
Donald E Kohan (donald.kohan@hsc.utah.edu)

Version: 5 Date: 20 November 2012

Author's response to reviews: see over
November 20, 2012

Dr. Hayley Henderson
Executive Editor
The BioMed Central Editorial Team

Dear Dr. Henderson:

Please find attached our revised manuscript (MS: 1059714313806168) entitled “Disruption of the endothelin A receptor in the nephron causes mild fluid volume expansion” by Stuart et al for consideration for publication in BMC Nephrology. We appreciate the reviewers’ thoughtful comments and have now revised the manuscript in response to these. Our itemized responses are below and changes in the text are indicated by red font.

Thank you for consideration of our work.

On behalf of the authors

Donald Kohan

Referee 1:

This study evaluates the effect of the genetic disruption of endothelin A receptor on blood pressure and salt handling in mice with doxycycline-induced nephron specific deletion of ETA receptor. The authors conclude that this genetic manipulation leads to a mild fluid retention suggesting a minor role of nephron ETA receptors in sodium handling under physiological conditions.

Major comments:

The authors should discuss the changes in body weight and decreasing hematocrit in view of unchanged extracellular fluid and total body water under high salt intake. What was the percentage change of body weight during the experiment?

Response: The percent changes in body weights are now shown in the results section. We also discuss the changes in body weight and decreasing hematocrit in view of unchanged extracellular fluid and total body water under high salt intake in the first paragraph of the discussion.

Minor comments:

1. Were male or female used in the experiments? There could be different effects on male and female on sodium handling as has been previously shown in rats. Discussion to this topic should be added.

Response: Equal numbers of males and females were used – this is now indicated in the methods. There were no gender-based differences in any of the observed parameters – this is now indicated in the results section.

2. In the statistical analysis, it is stated that mean percent of control were analyzed, however also absolute values (Fig 5 and 6) were used in the study-please correct this.

Response: Thank you for catching this – it has now been corrected in the methods.
section description of the statistical analysis.

3. Figure 4-the description of experimental schedule is different in figure and methods section. In the figure, it should be better to number the days following doxycycline treatment sequentially, i.e. 21, 22 etc in order to highlight that it is the continuation of the experiment.

Response: Thank you – we now have the figure and methods being the same – mice are given a normal salt diet for 3 days and a high salt diet for 7 days, both before and after DOX administration. We have changing the numbering of days as suggested.

4. Fig 6-the authors should explain why different time schedule (7+7 days instead of 3+7) for the experiments with body volume status were used.

Response: We now indicate in the methods that we did the 7 days of normal Na diet and 7 days of high Na diet for body volume status studies in order to help insure that the animals had achieved maximal volume status stability. The BP studies are done continuously with no anesthesia during the analysis, so 3 days of normal Na diet is plenty (they have, in fact, been on a normal Na diet all along prior to the study). The metabolic cage studies are also done without anesthesia with no major disturbance of the mouse. In contrast, the body volume studies required drawing blood and anesthesia for the impedance analysis, so we wanted to give them as much time as was reasonable to achieve a steady state.

5. Fig.6-the explanation of statistical significances is missing. SI units use kilograms (kg) for weight.

Response: Thank you – we have corrected this. We are unsure what the referee means by SI units using kg – we report all data in this figure as grams (g), which is the standard way of reporting these values in mice.

Referee 2:

The present study investigated the role of renal epithelial ETA receptors for sodium and fluid homeostasis using a tissue-specific inducible knock out model. The study shows that renal epithelial ETA-R knock out does not lead to increased sodium sensitivity of arterial pressure but causes modest volume retention under conditions of high NaCl intake based on body weight, impedance and hematocrit measurements.

Two minor essential revisions

The authors conclude that ETA-R disruption in the nephron causes mild Na+ retention. The difficulty to detect subtle differences in Na+ and water balance by metabolism cage studies in mice is acknowledged. The metabolism cage protocol did not allow detecting transient changes in sodium and water balance in response to the switch from normal to high NaCl diet. Was water and sodium intake measured for calculation of respective balances? If no direct evidence for increased Na+ retention in response to renal epithelial ETA-R knockout can provided so far, it is suggested to substitute Na+ retention by volume retention in the conclusion.

Response: Water and Na intake was assessed and there were no differences in these between mouse groups, although water and Na intake increased when mice were placed on a high Na diet as expected. We now describe this in the results.

The legend of Fig. 6 should indicate what statistically significant difference the asterisks indicate to facilitate reading.

Response: Thank you – this has been corrected.
Referee 3:

The manuscript by Stuart et al. studies the role of ETA receptors in the nephron on renal function and blood pressure regulation. The authors generated a conditional (doxycycline-inducible) nephron specific KO for the ETA receptor and measured several renal parameters and total body and extracellular fluid content in mice on normal or high salt. The authors report minimal changes in excretory function and no change in blood pressure. Nephron ETA KO have a significant increase in body weight on high salt and a tendency towards an increase in extracellular fluid. There is a significant decrease in hematocrit during high salt, suggesting hemodilution due to enhanced water retention.

Major Comments

1. Is ETB mRNA in the nephron affected by nephron ETA KO?. This could be done in the same mRNA samples isolated from PT, TAL and CCD. An increase in ETB could explain the lack of effect of nephron-ETA in renal function.

   Response: Thank you, this is an excellent point. We have now measured ETB mRNA in these mice in microdissected PT, TAL and CCD. As described in the results, we detected no differences in ETB mRNA between ETA KO and control mice in these nephron segments.

2. The authors need to provide a rational explanation why they think there is an increase in body water when there is no change in Na excretion. If something there is a tendency for ETA KO to loose more volume (albeit not significant).

   Response: We now explain this in the first paragraph of the discussion. In our experience, urinary Na excretion measurements in mice have very high variability, making detection of significant differences between mouse groups very challenging. The only time we have detected significant differences in urinary Na excretion between groups was when such differences were quite large, unlike in the current study where the fluid retention is quite small. Indeed, if you calculated the amount of Na retained that would cause the fluid retention we observe, it would be too small to detect unless there was almost no standard error.

3. While most data are negative and show a small effect of nephron ETA the scientific approach to study this is extremely straightforward and most appropriate to study gene function in nephron physiology. The Pax8/inducible Cre mice should be of high value to the renal community.

   Response: Thank you, we agree.