Reviewer’s report

Title: Loss of ectonucleotidases from the coronary vascular bed after ischemia-reperfusion in isolated rat heart

Version: 1 Date: 24 February 2013

Reviewer: Florian Bönner

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The study by Takahashi-Sato et.al. investigates nucleotide turnover of endothelial ectonucleotidases in a model of isolated perfused hearts according to the Langendorff protocol with HPLC and immunoblot analysis. They propose that functionally intact ectonucleotidases are liberated in response to ischemia from coronary endothelium and this may serve as novel markers for reperfusion induced injury to the heart. This effect was pronounced in aged rats.

This is an elegant study, showing shedding of functionally intact ectonucleotidases into coronary circulation after the cell-stress of ischemia. The authors used exogenous applied nucleotides (Etheno-Nucleotides) to eliminate the bias of cytosolic nucleotides (e.g. from ischemic cells) to determine activity of coronary endothelial ectonucleotidases after ischemia.

Major comments:

(1) Why were basal measurements of nucleotides in coronary perfusate effluent not possible? There is good evidence that nucleotide release can be measured with HPLC even under basal conditions (Deussen, Weichsel et al., 2006).

(2) The authors used a setting of constant flow and thus varying pressure. The authors have to make sure by presenting representative data, that the pressure was high enough all the time to establish a sufficient coronary perfusion. Constant flow models are prone to biasing effects of mal-perfusion in coronary microcirculation due to low pressure.

(3) In the latter regard, please discuss the observation of increased perfusion pressure after ischemia. This is absolutely untypically, especially when high amounts of adenosine are measurable, which would lead to vasodilatation and thus a drop in pressure.

(4) Ischemia was induced by disconnecting the flow. A better method would be to switch to buffer at low O2 pressure (Borst and Schrader, 1991). This has the unique advantage that the heart will not dry out during ischemia. Again: Was there significant perfusion of the microcirculation after “dry” ischemia? Where there any sign of coronary obstruction due to drying?

(5) It is known that CD39 molecule density on coronary endothelium decreases in case of ischemia and reperfusion injury in mice. The mechanism (downregulation, re-uptake, shedding) remained unclear so far (Bonner, Borg et al., 2012). This could be touched on in the discussion section.
(6) The meaning and legend of figure 6 is unclear. There is the impression, that CD39 and CD73 are constitutively shedded already under pre-ischemic conditions. Please revise the figure.

(7) To draw the conclusion, that the liberation of CD39 and CD73 would be a marker of reperfusion injury might be a little “straight forward”. 30 Minutes of global ischemia is very mild and may not lead to great vascular or even cardiomyocyte damage (Redel, Jazbutyte et al., 2008). However, this finding is of significance, because this might be a reaction of coronary endothelium after episodes of brief ischemia.

(8) To draw the conclusion of post ischemic cardiac function to be improved by inhibition of the observed processes might also be a “little straight forward”, since this was not addressed in the study and would need further clarification by experiments.

(9) When there were nucleotide converting enzymes, functionally active within the effluvate, would’nt it be nice to see their lack in coronary circulation.

Minor comments:

a. Replace „heart“ by „coronary circulation“ (page 15, line 6)
b. Delete “an” in page 17, line 4

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests