98

Molecular dynamics and functional studies suggest brachyNH2 by binding to argininosuccinate synthase, induces relaxation in rat small mesenteric arteries

Daniel Arcanjo[1,2], *Isadora Bezerra*[2], *Daniel Moreira*[3], *Simon Gabriel Comerma Steffensen*[1], *Andreanne Vasconcelos*[3], *Judit Prat-Duran*[1], *Catia Teixeira*[4], *Paula Gomes*[4], *Ana Carolina Cardoso-Teixeira*[5], *Andrelina Coelho-de-Souza*[5], *Marcelo Bemquerer*[6,9], *Ms Birgitte Christensen*[1], *Reinhard Wimmer*[7], *Aldeidia Oliveira*[2], *Ricardo Ramos*[8], *Ulf Simonsen*[1], *Jose Roberto Leite*[3]

[1]Aarhus University, Aarhus, Denmark, [2]Federal University of Piaui, Teresina/Piaui, Brazil, [3]University of Brasilia, Brasilia/Brasilia, Brazil, [4]Universidade do Porto, Porto, Portugal, [5]State UNiversity of Ceará, Fortaleza, Brazil, [6]Embrapa Genetic Resources and Biotechnology, Brasilia, Brazil, [7]Aalborg University, Aalborg, Denmark, [8]Federal Institute of Piaui, Teresina, Brazil, [9]Embrapa Dairy cattle, Juiz de Fora, Brazil

Introduction: Previous studies have reported the involvement of the argininosuccinate synthase (AsS) as a putative target for cardiovascular effects of proline-rich oligopeptides. The present study investigated the mechanisms underlying the vasorelaxant effect of BPP-BrachyNH2 in rat small mesenteric arteries and applied in silico molecular dynamics studies to investigate the interaction between AsS and BPP-BrachyNH2.

Method and Material: Segments of arteries with internal diameters of 200-250 µm from male Wistar rats (300-350 g) were mounted in microvascular myographs being treated with lipopolysaccharide (LPS), and then, BPP-BrachyNH2 was cumulatively added to noradrenalin-contracted preparations. Immunochemistry was done for AsS, endothelial nitric oxide (NO) synthase (eNOS), and inducible nitric oxide synthase (iNOS) in these preparations. Molecular Dynamics calculations were performed between AsS enzyme as the target and both BPP-BrachyNH2 and L-citrulline as ligands.

Results: Immunohistochemistry showed expression of AsS, eNOS in mesenteric arteries, and of iNOS in segments exposed to LPS. The vasorelaxant effect of BPP-BrachyNH2 was abolished in the presence of L-NOARG and ODQ, and attenuated in the presence of 1400W and MDLA, an inhibitor of AsS. Besides, the AsS_BPP-BrachyNH2 complex showed increased binding energy, inhibition constant and number of interactions with aminoacids when compared with the AsS_L-citrulline complex.

Conclusion: These results suggest the positive interaction of BPP-BrachyNH2 with AsS, leading to L-citrulline recycling and increase of L-arginine bioavailability, a substrate for NO synthesis, as a mechanism for vasorelaxant effect. Our findings open new perspectives for potential therapeutic applications of proline-rich oligopeptides in NO-related vascular dysfunction.

99

Understanding and extending the Landis experiment

Jordan M. Petrow[1]

[1]University of Rostock, Faculty of Mathematics and Natural Sciences, Rostock, Germany

The Starling equation can be used to calculate the Landis experiment with the single capillary when that capillary is incorporated into a reservoir containing nutrient solution. However, the mathematics of the equation agrees with the design of the experiment only if the reservoir is open, the walls of the reservoir have a constant height, and if at the capillary the filtered amount of liquid would be slightly above its reabsorption. The first extension of the Landis experiment in the form that the walls of the reservoir are of unrestricted height promptly invalidates the validity of Starling's equation: (1) The imitated "interstitial fluid pressure" (IFP) is no longer an independent parameter in the equation in the experiment with the first extension. (2) The IHP is now a product of the blood pressure, IFP= F(PC), because it depends on the height of the filtered fluid column in the reservoir. (3) Via the function F(PC), the blood pressure PC is involved in reabsorption at the capillary wall itself. In the second extension of the Landis experiment, the reservoir with the capillary is closed with the result that Starling's equation is finally rejected as the basis of the capillary calculation. This subject will be dealt with in another paper.