Mouse mucosal mast cell protease 4 generates endothelin-1 *in vitro* and *in vivo*

Martin Houde[1], Julie Labonté[1], Marc-David Jamain[1], Gunnar Pejler[2], Michael Gurish[3], Shinji Takai[4], Pedro D'Orléans-Juste[1]. [1]Université de Sherbrooke, Département de Pharmacologie, J1H 5N4, Canada, [2]Swedish University of Agricultural Sciences, Department of Anatomy, Biology and Biochemistry, SE-750 07, Sweden, [3]Brigham and Women's Hospital, Department of Rheumatology, 02115, United States, [4]Osaka Medical College, Department of Pharmacology, 569-8686, Japan.

The serine protease chymase converts intravenously (*iv*)- administered big-endothelin-1 to endothelin-1 (ET-1) in the mouse *in vivo* (Simard *et al*., 2009). We have subsequently reported a significant reduction of the pressor response to iv-administered big-ET-1 in mouse mucosal mast cell protease 4 knock out (-/-) mice (mMCP-4 KO), when compared to wild type (WT) congeners (APS-ET-11 International Conference, Montreal, 2009).

In the present study, the contribution of mMCP-4 was determined in the conversion of exogenous big-ET-1 *in vitro* and *in vivo* as well as in the tissue production of endogenous ET-1. Quantitative real time PCR revealed no significant differences in mRNA levels of neutral endopeptidase 24.11, mMCP-1, endothelin converting enzyme 1a and carboxypeptidase A in the cardiac left ventricle, aorta, kidney and lungs homogenates derived from wild type (WT) or mMCP-4 KO mice. Big-ET-1 (0.5 nmol/kg, *iv*), on the other hand, triggered a significantly higher plasma levels of immunoreactive ET-1 in WT mice when compared to mMCP-4 KO mice, (WT: 31.24 ± 5.26; mMCP-4 KO: 5.65 ± 0.68 fmol/ml of plasma; $p<0.001$, n=6). In addition, a chymase inhibitor-sensitive hydrolysis of the fluorogenic peptide Suc-Leu-Leu-Val-Tyr-7-amino-4-methylcoumarin (MCA) was detected in supernatants derived from pulmonary, aortic, left cardiac ventricular as well as cortex and medullar renal homogenates of WT but not mMCP-4 KO mice (WT *vs*. mMCP-4 KO: $p<0.05$ n=6 different experiments for each group). Big-ET-1 (1 µM) incubated for one hour in supernatants derived from pulmonary homogenates of WT, but not mMCP-4 KO mice, generated HPLC-detectable levels of ET-1 (1-31) with no further hydrolysis towards ET-1. Finally, tissue levels of immunoreactive ET-1 were found to be reduced by more than 40 % in whole lung homogenates from mMCP-4 KO mice when compared to those from WT congeners ($p<0.05$, n=5).

We conclude that mMCP-4 is the predominantly involved chymase isoform in the production of endogenous endothelin-1 in the mouse model.

Supported by the Canadian Institutes of Health Research and the Fonds de la Recherche en Santé du Québec.