NEUROPEPTIDES IN CARDIOPROTECTION AND CARDIOVASCULAR DISEASES

PhD thesis

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1. Introduction

1.1. Neuropeptides and the cardiovascular system

Neuropeptides play an important role the physiology and pathology of the cardiovascular system. Some of them are considered to exert in general rather beneficial or protective effects e.g. SST, while others e.g. SP and NPY also have a detrimental role in various cardiovascular diseases.

1.2. Somatostatin in ischemia reperfusion injury

Although, ischemic heart diseases belong to the leading causes of death worldwide, there are still no effective marketed medications exerting cardioprotective effects against ischemia-reperfusion injury. Therefore, there is an unmet need for developing novel, cardioprotective drug candidates. Despite that the administration of synthetic SST analogs exert protective effect against ischemia/reperfusion injury of retina, liver, pancreas, and heart the expression and function of SST in ischemia/reperfusion injury of the heart and its involvement in cardioprotection is not known.

1.3. DPP4 inhibition, Substance P and Neuropeptide Y in heart failure

In connection to the detrimental effects of neuropeptides the SAVOR-TIMI 53 phase 4 clinical trial revealed that in the saxagliptin -treated patients the rate of hospitalization for heart failure is increased but the mechanism of this side effect is not clarified. We hypothesized that the neuropeptide-type substrates of the DPP4 (SP and NPY) may play a role in the adverse effect of saxagliptin**.**

2. Objectives

Although, ischemic heart diseases belong to the leading causes of death worldwide, there are still no effective marketed medications exerting cardioprotective effects against ischemia-reperfusion injury. Therefore, there is an unmet need for developing novel, cardiac tissue protective drug candidates. In order to test the potential cardioprotective/citoprotective effect of SST a relatively quick and efficient first step is the utilization of the *in vitro*, cell culture-based simulated ischemia-reperfusion injury model. Next, to

provide further evidence supporting the protective role of SST we used tissue and plasma samples obtained from our previous, translationally relevant pig myocardial infarction and cardioprotection study where we thoroughly tested the protective effects of different ischemic conditioning interventions. Briefly, in this model we have found that myocardial necrosis is significantly decreased by ischemic preconditioning, meanwhile myocardial edema is reduced by ischemic postconditioning and remote ischemic conditioning, and microvascular obstruction is rather decreased by ischemic preconditiong and ischemic postconditioning. To further improve the translational relevance of the current results we performed investigations on cardiac tissue samples of healthy patients or patients with chronic heart failure.

The results of the neuropeptide research of the last 2-3 decades highlighted that various neuropeptides may play a role both in protective cardiovascular mechanisms and pathological processes of cardiovascular diseases and their co-morbidities. The SAVOR TIMI 53 clinical trial revealed that DPP4 inhibition by saxagliptin-treatment increased HF-associated hospitalization of patients with chronic HF. We assume that this harmful effect is at least partially mediated by neuropeptide substrates of DPP4 (NPY and SP). Therefore, the major objectives of this work were:

I. First, to investigate the direct cardiocytoprotective effect of SST against simulated ischemia/reperfusion injury in cardiac cell cultures.
II. Next, to investigate the expression of SST and its receptors in pig and human hearts.
III. In addition, to determine the expression of DPP4 and its substrates in the human heart and cell culture samples both at protein and mRNA levels.
IV. Finally, to set up a relevant cell culture platform to investigate the potentially detrimental effect of DPP4 inhibition by saxagliptin and the role of two important neuropeptide substrates of the DPP4 enzyme such as SP and NPY.

3. Methods

We have purchased AC16 and H9C2 cell lines from international vendors and cultured them according to the official recommendations of the provider company and the broadly accepted rules of the cell-culturing practice (humidified atmosphere of 5% CO_2 at 37 °C, medium: DMEM/F12 and DMEM with 10% FBS).

Primary adult rat cardiomyocytes were isolated from anesthetized male Wistar rats and separated by collagenase II digestion and maintained in M199 medium. In order to prepare the co-culture of neonatal rat cardiac fibroblasts and myocytes neonatal rat hearts were isolated from 1–3-day-old Wistar rats and the cells were maintained in DMEM medium supplemented with 1% FBS.

To perform the *in vitro* simulated ischemia/reperfusion injury protocol cells were treated with either SST or its solvent containing growth media afterward cells were exposed to the combination of hypoxic solution and hypoxic gas atmosphere in a three-gas incubator (95% N_2 and 5% CO_2). After the exposure to hypoxic conditions cells were placed back to normal maintenance conditions to simulate the effects of reperfusion. At the end of the reperfusion period cell viability was measured using calcein assay.

In the scratch assay-based experiments cells were pretreated with saxagliptin or its solvent. Afterwards the cell monolayer was scratched and the cell debris was removed by washing with Hank's Balanced Salt Solution. Cells were then treated with a combination of saxagliptin and NPY or SP. Images were taken at the start of the treatment (0 h) and 24 h after the scratching process. The wound area of the images was measured by ImageJ software and expressed as the percentage of baseline value (0 h).

In situ hybridization of DPP4 enzyme, SSTR1-2, and cell-type specific markers' mRNA was performed using RNAscope Multiplex Fluorescent Kit v2 according to the manufacturer's instructions on formalin-fixed paraffin-embedded tissue slides of human control hearts. Tissue sections were pretreated with heat, H_2O_2, and protease before hybridization with the various target oligo probes. Preamplifier, amplifier, and HRP-labeled oligo probes

were then sequentially hybridized, after which signal development was performed with TSA fluorophores (Cy3, FITC). Cell nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI) and imaging was performed with a Leica DMI8 Confocal microscope.

To investigate whether DPP4 expression was altered at the protein level in human hearts, Western blot experiments were performed. Frozen tissue samples from the interventricular septums were homogenized with a TissueLyser in RIPA containing HALT Protease and Phosphatase Inhibitor. Protein concentration of the samples was measured using the bicinchoninic acid assay kit. Equal protein amounts from each sample were mixed with Laemmli buffer with β-mercaptoethanol and were loaded on Tris-glycine sodium dodecyl sulfate-polyacrylamide (4–20%) gels. After electrophoresis-based separation, proteins were transferred onto polyvinylidene difluoride membrane with Trans-Blot® TurboTM Transfer System. The membranes were blocked in 5% BSA in Tris-buffered saline with 0.05% Tween-20. Next, the membranes were incubated overnight with anti-DPP4 primary antibody. Next, three washes were performed in TBS-T, and then membranes were incubated with HRP-conjugated anti-rabbit secondary antibodies and washed in TBS-T. Signals were detected after the incubation with enhanced chemiluminescence kit by Chemidoc XRS+. Image analysis was performed with the Image Lab™ 6.0 software. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as loading control. Membranes were incubated with Restore Stripping Buffer, and then incubation with anti-GAPDH primary antibody, and HRP-conjugated anti-rabbit secondary antibody were performed. Afterwards signals were detected.

To determine the SP-like immunoreactivity in human interventricular septum the samples were homogenized in phosphate buffer and protease inhibitor with tissue homogenizer device. Next, centrifugation was performed and the supernatant was collected and pooled at −80 °C. SP-like immunoreactivity was measured by a specific and sensitive radioimmunoassay method using the substance P competitive radioimmunoassay kit. Reconstituted positive controls and standards, 100 mL of tissue homogenates in duplicates, and 100 µL antiserum were incubated overnight. Next, ^{125}I-labelled SP tracer was

added to the tubes on the following day and another overnight incubation was performed. Goat anti-rabbit IgG serum and normal rabbit serum were added to the designated tubes on the next day and incubated. Centrifugation was performed to collect immunocomplexes, supernatant was carefully discarded, and pellet cpm was determined by g-counter. The results were expressed as fmol SP-like immunoreactivity per mg total protein weight.

Determination of the SST-like immunoreactivity in the cardiac and plasma samples was performed similarly as it is described in the previous paragraph. Only the differences in the methodology are highlighted here. Tissue samples were homogenized in distilled water containing protease inhibitor. Protein extraction was performed from 1 ml of the plasma samples with the mixture of absolute ethanol and 96% acetic acid. Tubes were incubated followed by a centrifugation. Afterwards, the supernatant was collected into a reaction tube and it was dried under a nitrogen flow. Next, it was resuspended in the assay buffer and centrifuged before radioimmunoassay. According to the aim of the measurement, ^{125}I-labeled somatostatin-14 tracer was used and the antiserum was raised in sheep against somatostatin-14-bovine thyroglobulin.

Determination of NPY levels in human interventricular septum samples was performed using RayBio® Human/Mouse/Rat Neuropeptide Y competitive Enzyme Immunoassay Kit following the manufacturer's instructions. First, the anti-NPY antibody solution was added to each well and then incubated with gentle shaking at room temperature. Next, the solution was removed and the wells were washed with wash buffer solution, which was repeated four times. After the last wash, the wash buffer was removed and the plate was inverted and blotted against clean paper towels. Afterwards, 100 µL of standard reagents, positive control, and samples were added to the appropriate wells. The wells were covered and incubated overnight with gentle shaking. Next, the solutions were removed, and the wells were washed four times with wash buffer solution. HRP-streptavidin solution was added and the plate was incubated with gentle shaking. After the following washing step, 100 µL of TMB One-Step Substrate Reagent was added and it was with gentle shaking, while protecting the assay from light. At the end of the incubation, stop solution was added and color intensity measurement was performed with

Labsystems DC plate reader. Results were expressed as ng of NPY/mg of total protein content. Total protein concentration was measured with a bicinchoninic acid assay kit using bovine serum albumin as a standard.

Total RNA from porcine left ventricle samples (ischemic zone) was isolated with Direct-Zol RNA Mini Prep following the manufacturer's instructions. RNA was treated with DNase I, and RNA concentration was measured by spectrophotometer. 1 μg of total RNA was reverse transcribed into complementary DNA with Maxima™ First Strand cDNA Synthesis Kit for real-time quantitative PCR (RT-qPCR).

The expression of SST and SSTRs was assessed with Biometria TProfessional Basic Gradient PCR equipment. Identification of PCR products were performed by agarose (2%) gel electrophoresis.

Relative gene expression ratios were measured by Stratagene Mx3000P QPCR System. β-ACTIN was used as a reference gene based on pilot experiments and literature data. Amplification of target genes were carried out using Luminaris HiGreen Low ROX qPCR Master Mix. Dissociation curve analysis were also performed.

Human heart samples were lysed in 1 ml QIAzol Lysis Reagent. Total RNA was extracted from the lysates with Direct-Zol RNA Mini Prep System with DNase I treatment according to the manufacturer's protocol. The RNA integrity numbers and RNA concentration were measured by RNA ScreenTape system with 2200 Tapestation and RNA HS Assay Kit with Qubit 3.0 Fluorometer, respectively.

For Gene Expression Profiling library construction, QuantSeq 3' mRNA-Seq Library Prep Kit FWD for Illumina was applied following the manufacturer's protocol. The quality and quantity of the library were measured by using High Sensitivity DNA1000 ScreenTape system with 2200 Tapestation and dsDNA HS Assay Kit with Qubit 3.0 Fluorometer, respectively. Pooled libraries were diluted to 1.8 pM for 1 × 86 bp single-end sequencing with 75-cycle High Output v2.5 Kit on the NextSeq 500 Sequencing System using the manufacturer's protocol. RNA-sequencing datasets of the human samples are deposited in the ArrayExpress database (accession number: E-MTAB-10720).

Out of this data set, here, we present the SST and SSTR1-5 genes-related sequencing data from control human cardiac samples.

mRNA-sequencing data of left ventricle samples of pig myocardial infarction model were analyzed. Alignment of the sequencing reads to the Sus scrofa reference genome assembly (Swine Genome Sequencing Consortium Sscrofa10.2/susScr3 UCSC), feature counting for the corresponding reference annotation, and the statistical analysis of differential expression were performed by the TopHat-Cufflinks workflow. In this analysis, TopHat version 2.1.1, Bowtie2 version 2.2.3, and Cufflinks version 2.2.1 were used.

Raw reads determined by RNA-sequencing of the human samples were preprocessed by Cutadapt (version 1.15). During this step adapters, the poly(A) tail and bases below Phred score 30 were trimmed, and reads with a length of less than 19 nt were filtered. Quality control was performed using the FastQC (version 0.11.8) and MultiQC (version 1.7) software. HISAT2 (version 2.0.4), featureCounts (version of Subread 2.0.0), and DESeq2 (version 1.10.1) were utilized for alignment, annotation, normalization, and differential expression analysis, respectively. Homo sapiens Ensembl GRCh37 reference genome and annotation were applied for the analysis of the human samples. Differential expression analysis was performed by the DESeq2 software package.

GO enrichment analysis (database version released on August 10, 2020) was conducted for each of the possible comparison of the experimental groups. In order to obtain GO biological process terms enriched among differentially expressed genes compared to the Sus scrofa reference gene list, online PANTHER Overrepresentation Test [geneontology.org, version released on July 28, 2020 was used. Enrichment analysis was performed by applying Fisher's exact test with false discovery rate adjustment for multiple comparisons.

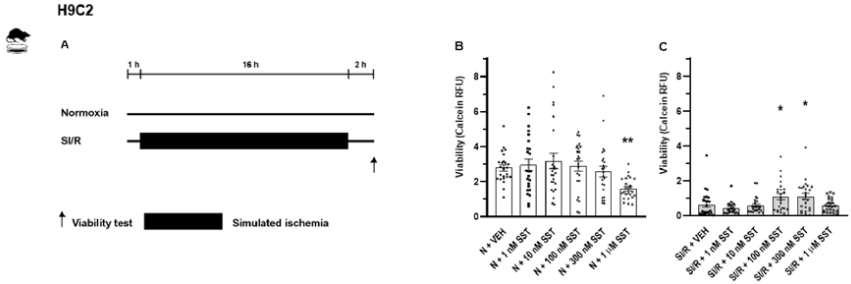
Statistical analyses and Rout outlier analyses were performed and graphs were created using GraphPad Prism version 8. Kruskal-Wallis test with Dunn's post hoc test, one-way analysis of variance (ANOVA), two-way ANOVA, unpaired t-test, and Mann–Whitney test were used to find statistically

significant differences. Those were considered significant at values of $p < 0.05$. Unless noted otherwise, all data represent the mean ± SEM. In order to avoid the possibility of overlooking significant differences due to small group sizes, ANOVA-like nonparametric bootstrap-based comparison of means with 1,000 times resampling was also performed on relative expression ratios assessed by RT-qPCR.

4. Results

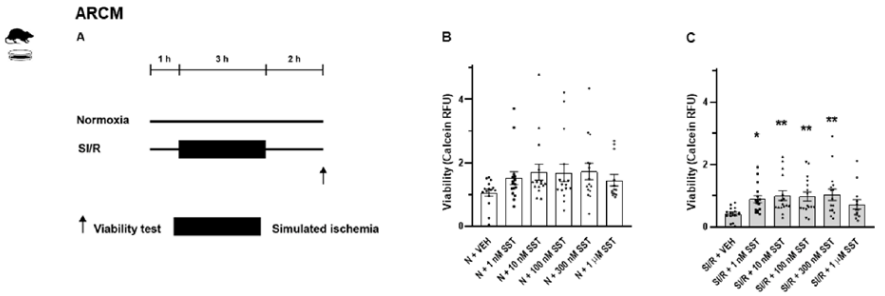
In order to investigate the potential cardiocytoprotective effect of SST, we performed *in vitro* simulated ischemia/reperfusion experiments in H9C2 cells and adult rat cardiomyocytes, respectively. Simulated ischemia/reperfusion significantly decreased both the viability of the H9C2 cells and adult rat cardiomyocytes compared to normoxic controls.

- We have found that the simulated ischemia/reperfusion-induced cell death was attenuated by SST treatment at 100 and 300 nM showing a concentration-dependent, bell-shaped cardiocytoprotective effect in H9C2 cells. SST did not alter the viability of normoxic cells, except for the highest concentration of SST (1 μM) that caused a significant cell death in H9C2 cells.



- SST also shows a concentration-dependent, bell-shaped cardiocytoprotective effect against simulated ischemia/reperfusion-induced injury in adult rat cardiomyocytes where SST treatment at 1, 10, 100, and

300 nM concentrations increased the viability significantly. Although the cell viability shows a similar dose-response pattern in the SST-treated groups in normoxic conditions, these changes were not statistically significant.

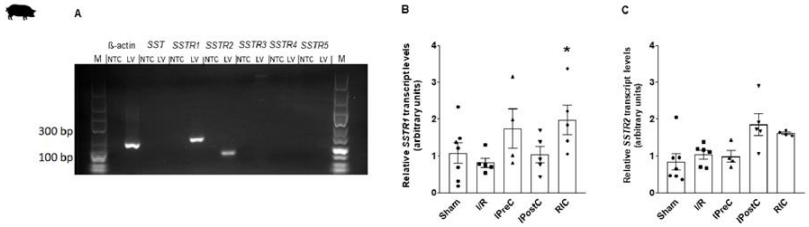


After we have demonstrated that the native SST exerts a direct cardiocytoprotective effect *in vitro*, we have aimed to further support the protective role of SST with data obtained from a translational porcine model of myocardial infarction and cardioprotection, and to investigate the expression of various SST receptors. Accordingly, we used tissue and plasma samples collected in our previous porcine myocardial infarction and cardioprotection study with different ischemic conditioning interventions. In order to determine SST-like immunoreactivity in pig plasma and left ventricle samples, we performed radioimmunoassay experiments.

- We detected SST-like immunoreactivity in pig left ventricle samples, but we found no differences between groups. The plasma SST-like immunoreactivity increased significantly in ischemic preconditioning samples compared to the sham group.

Next, we aimed to detect *SST* and its receptor mRNA expression in pig left ventricle, therefore we performed RT-qPCR and bioinformatics analysis of mRNA-sequencing data. In addition, we performed a bootstrap-based comparison of means to confirm the RT-qPCR results.

- We detected the expression of *SSTR1* and *SSTR2* mRNA by PCR, but not *SST* and its other receptors (*SSTR3*, *SSTR4*, and *SSTR5*).
- The results of the bioinformatics analysis of mRNA-sequencing data confirmed all these results. We identified a significantly upregulated relative expression of *SSTR1* in the remote ischemic conditioning group compared to the ischemia/reperfusion group. There were no significant differences in *SSTR2* mRNA expression between any groups. The bootstrap analysis also confirmed these results.



In order to determine SST-like immunoreactivity also in human interventricular septum samples, we performed radioimmunoassay experiments in cardiac samples of both healthy patients and patients with ischemic cardiomyopathy.

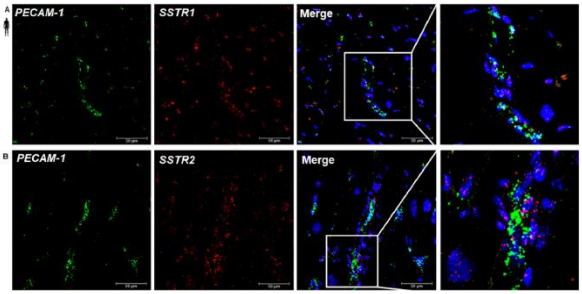
- We measured a significant decrease of tissue SST-like immunoreactivity in ICM samples compared to the control group.

To detect *SST* and its receptor mRNA expression in human left ventricles we performed bioinformatics analysis of the mRNA-sequencing data.

- We detected the expression of the mRNA of *SSTR1, SSTR2,* and *SSTR5* receptors, but not *SST* and its other receptors (*SSTR3* and *SSTR4*).

In order to determine the SSTR expression more precisely we performed RNAscope *in situ* hybridization assay for the mRNA of *SSTR1 and SSTR2* in human healthy left ventricular samples.

- We found that expression of the mRNA of both *SSTR1* and *SSTR2* are localized primarily in *PECAM-1* mRNA-positive endothelial cells; however, both *SSTR1* and *SSTR2* mRNA were detected in other cell types, including *TAGLN* mRNA-positive vascular smooth muscle cells and *RYR2* mRNA-positive cardiomyocytes. There was no detectable signal on the negative control slides.

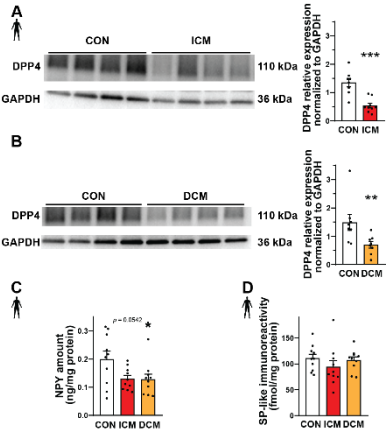


To identify the biological processes those could contribute to the cytoprotective effect of SST, SSTR1 and SSTR2 we performed the Gene Ontology (GO) analysis of all differentially expressed mRNAs for each possible comparison of the experimental groups. The top thirty results of the comparison between remote ischemic conditioning and ischemia/reperfusion groups—where *SSTR1* expression significantly increased—are presented here.

- Differentially expressed mRNAs were significantly associated with, e.g., cardiac muscle differentiation, skeletal muscle development/regeneration, and response to oxidative stress.

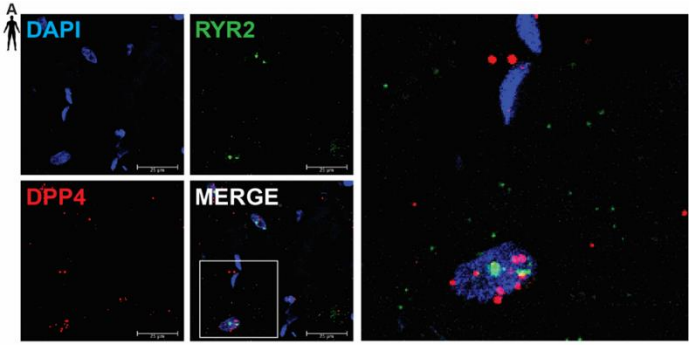
In the second study we aimed to determine first the expression of DPP4, NPY and SP at the protein level, therefore we performed Western blot, enzyme-linked immunosorbent assay (ELISA), and radioimmunoassay experiments in interventricular septum samples of failing human hearts (ischemic cardiomyopathy (ICM) and dilated cardiomyopathy (DCM)), and also healthy control (CON) hearts.

- We identified a significantly decreased expression of DPP4 both in the ICM and DCM samples compared to the healthy hearts. NPY expression significantly decreased in the DCM samples and the ICM samples showed a tendency of decrease compared to the control. The expression of SP remained on a similar level in each group.



Next, we aimed to determine the cell-type-specific DPP4 expression, therefore RNAscope *in situ* hybridization assay was performed on left ventricular tissue slides of healthy humans.

- We have found that the mRNA of *DPP4* is primarily expressed by ryanodine receptor 2 (*RYR2*) mRNA-positive cardiomyocytes. We have also detected DPP4 expression to some extent in platelet endothelial cell adhesion molecule 1 (*PECAM-1*) mRNA-positive endothelial cells, but not in vimentin (*VIM*) mRNA-positive fibroblasts and CD68 mRNA-positive macrophages. There was no detectable signal on the negative control slides. These observations indicate that DPP4 is primarily expressed in cardiomyocytes and endothelial cells in the heart tissue.

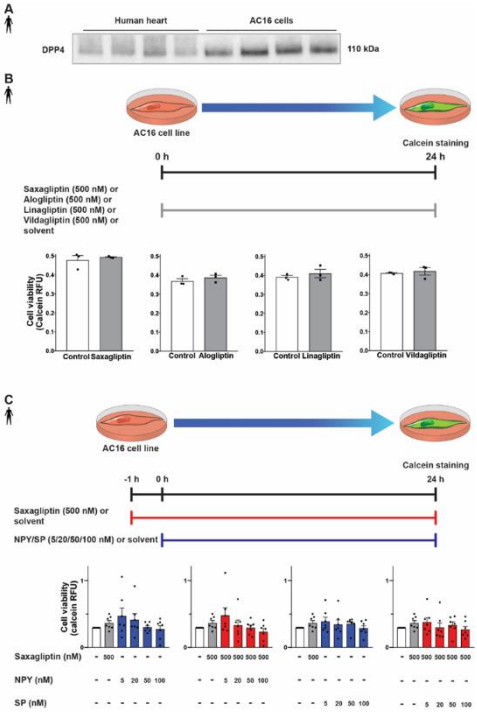


After we successfully determined the expression profile of DPP4 and its substrates, next we tested the potential harmful effects of DPP4 inhibition and/or its substrates (SP, NPY) on cardiomyocytes. In order to begin the *in vitro* cell culture-based experiments, first an appropriate cell culture model was required. We chose the AC16 cell line since it expresses the DPP4 enzyme according to the results of the Western blot measurements, also confirming our RNAscope results. In the beginning, we hypothesized that direct cytotoxicity could be responsible for the increased hospitalization rate caused by saxagliptin, thus we performed calcein-based cell viability tests on AC16 cells treated with saxagliptin or other clinically relevant gliptins.

- We have found that DPP4 inhibition by any of the tested gliptins (saxagliptin, vildagliptin, linagliptin, alogliptin) alone, at a concentration of 500 nM, does not have a toxic effect on cell viability of AC16 cells.

Next, we shifted our focus to investigating whether decreased DPP4 enzyme activity by saxagliptin exerts cytotoxic effects in the presence of its substrates: NPY and SP. Thus, we treated the cells with NPY or SP or in combination with saxagliptin.

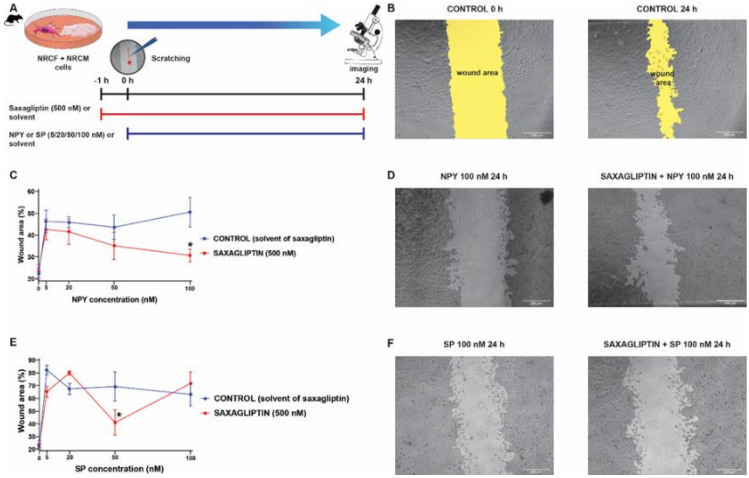
- We have found that neither neuropeptides (NPY or SP) alone nor in combination with saxagliptin decreases the viability of AC16 cells. These results indicate that both saxagliptin and the neuropeptide substrates of DPP4 do not influence the viability of healthy cells; thus, a different approach and model are required to clarify the potential cardiotoxic effect of saxagliptin.



According to the literature data SP and NPY potentially exert their harmful cardiovascular effects through the modulation of fibroblast activities. In line with these data and our results that DPP4 is expressed by cardiomyocytes, we continued our experiments with a co-culture model of primary neonatal rat cardiomyocytes and cardiac fibroblasts. We performed scratch assay (frequently used *in vitro* model of cell migration and wound healing) experiments with the same treatment protocol (neuropeptides and/or saxagliptin).

- We have found that administration of either NPY or SP decreases the migration speed of the cells significantly compared to the control groups

(CONTROL and SAXAGLIPTIN). At the highest concentration of NPY (100 nM), the administration of saxagliptin (500 nM) restored the migration capacity of fibroblasts. In the case of the SP administration, there was a significant but not a meaningful difference caused by saxagliptin.



5. Conclusions

We demonstrated the first time that native SST protects cardiomyocytes against ischemia/reperfusion injury. Interestingly, SST peptide is expressed in the heart; although, its mRNA is not detectable, therefore we suspect that it has sensory neural origin. SSTR1 and SSTR2 might be involved in the SST-mediated cardioprotective effects, but other mechanisms such as antioxidant activity cannot be excluded. These results highlight new aspects of SST and SSTR signaling on the pharmacological relevance in cardioprotection. We also showed that in human failing hearts DPP4 expression decreases and the neuropeptide tone changes. Interestingly, neither DPP4 inhibition nor its combined administration with neuropeptides caused cytotoxicity *in vitro*. However, NPY and SP inhibited the cell migration in a co-culture model of primary neonatal rat cardiomyocytes and cardiac fibroblasts. This effect of NPY was reversed by saxagliptin. We revealed that saxagliptin and NPY potentially interferes with cardiac tissue remodeling therefore contributing to the pathophysiology of end-stage chronic heart failure but the contribution of other, chemokine-type DPP4 substrates (e.g. RANTES, eotaxins, MDC, SDF-1) and immune-mediated processes could be significant, although we haven't investigated those is our study. We believe that the DPP4 enzyme could work as a compensatory mechanism against the altered neuropeptide tone caused by the elevated sympathetic activity in heart failure. Inhibition of DPP4 by saxagliptin potentially diminishes this adaptive mechanism, and thereby exacerbates myocardial damage. However, further investigations extended to additional DPP4 substrates are necessary to clarify more deeply their interaction with the long term adverse cardiac remodeling in HF and the exact background mechanisms.

6. Bibliography of the candidate's publications

6.1. Publications related to the candidate's Ph.D. dissertation

I. **Vörös, I.**, Onódi, Z., Tóth, V. É., Gergely, T. G., Sághy, É., Görbe, A., Kemény, Á., Leszek, P., Helyes, Z., Ferdinandy, P., & Varga, Z. V. (2022). Saxagliptin Cardiotoxicity in Chronic Heart Failure: The Role of DPP4 in the Regulation of Neuropeptide Tone. *Biomedicines*, *10*(7), 1573. https://doi.org/10.3390/biomedicines10071573

IF: 4.7

II. **Vörös, I.***, Sághy, É.*, Pohóczky, K., Makkos, A., Onódi, Z., Brenner, G. B., Baranyai, T., Ágg, B., Váradi, B., Kemény, Á., Leszek, P., Görbe, A., Varga, Z. V., Giricz, Z., Schulz, R., Helyes, Z., & Ferdinandy, P. (2021). Somatostatin and Its Receptors in Myocardial Ischemia/Reperfusion Injury and Cardioprotection. *Frontiers in pharmacology*, *12*, 663655. https://doi.org/10.3389/fphar.2021.663655

IF: 5.988 *equal contribution to this study

ΣIF of dissertation-related publications: 10.688

6.2. Publications independent of the candidate's Ph.D. dissertation

III. Kucsera, D., Tóth, V. E., Gergő, D., **Vörös, I.**, Onódi, Z., Görbe, A., Ferdinandy, P., & Varga, Z. V. (2021). Characterization of the CDAA Diet-Induced Non-alcoholic Steatohepatitis Model: Sex-Specific Differences in Inflammation, Fibrosis, and Cholesterol Metabolism in Middle-Aged Mice. *Frontiers in physiology*, *12*, 609465. https://doi.org/10.3389/fphys.2021.609465

IF: 4.755

IV. Róka, B., Tod, P., Kaucsár, T., Bukosza, É. N., **Vörös, I.**, Varga, Z. V., Petrovich, B., Ágg, B., Ferdinandy, P., Szénási, G., & Hamar, P. (2021). Delayed Contralateral Nephrectomy Halted Post-Ischemic Renal Fibrosis Progression and Inhibited the Ischemia-Induced Fibromir Upregulation in Mice. *Biomedicines*, *9*(7), 815. https://doi.org/10.3390/biomedicines9070815

IF: 4.757

V. Sághy, É., **Vörös, I.**, Ágg, B., Kiss, B., Koncsos, G., Varga, Z. V., Görbe, A., Giricz, Z., Schulz, R., & Ferdinandy, P. (2020). Cardiac miRNA Expression and their mRNA Targets in a Rat Model of Prediabetes. *International journal of molecular sciences*, *21*(6), 2128. https://doi.org/10.3390/ijms21062128

IF: 5.924

VI. Dora, D., **Vörös, I.**, Varga, Z. V., Takacs, P., Teglasi, V., Moldvay, J., & Lohinai, Z. (2023). BRAF RNA is prognostic and widely expressed in lung adenocarcinoma. *Translational lung cancer research*, *12*(1), 27–41. https://doi.org/10.21037/tlcr-22-449

IF: 4.0

ΣIF: 30.124