# Studies on the effector function of sensory neurons in experimental neuropathies

*Péter Horváth*

Thesis

University of Debrecen
Medical and Health Science Center
Department of Pharmacology
2006

# 1 List of abrevations

| | |
|---|---|
| B2m | beta 2- microglobulin |
| CGRP | calcitonin gene-releated protein |
| CGRPR | calcitonin gene-releated protein receptor |
| Ct | $C_T$ is defined as the number of cycles needed for the fluorescence signal to reach a specific threshold level of detection |
| DM | diabetes mellitus |
| DNS | desoxi-ribo - nucleid acid |
| DRG | dorsal root ganglia |
| ENG | elektroneurograf |
| EPO | erytroprotein |
| FPG | fasting plasma glucose concentration |
| FPI | fasting plasma inzulin concentration |
| HOMA | Homeostasis Model Assessment |
| HOMA-IR | Homeostasis Model Assessment insulin sensitivity (% |
| HOMA-%B | Homeostasis Model Assessment beta cell function (%B |
| i.c | intracellular |
| i.p. | intraperitoneli |
| L-NAME | NG- nitro- L-arginin metil ester |
| n. femoralis | Nervus femoralis |
| n.vagus | Nervus vagus |
| NaCl | Natrium-Clorid |
| NANC | non-adrenerg, non-cholinerg |
| NK1 | Neurokinin 1 receptor |
| NK2 | Neurokinin 2 receptor |
| PPT-A | Preprotachykinin-A |
| QRT-PCR | quantitative real-time polymerase chain reaction |
| RIA | radioimmunoassay |
| RNS | ribo – nukleic – acid |
| S.D. | Standard Deviation |
| S.E.M. | Standard Error of Means |
| SOD | super-oxid dismutase |
| SOM | somatostatin |

| | |
|---|---|
| SP | subtance P |
| SRIF I, II | somatostatin receptor family I, II |
| SSTR1, 4 | subtypes of Somatostatin receptor 1, 4. |
| STZ | streptozotocin |
| FS | field stimulation |
| TTX | tetrodototoxin |

# 2 Introduction

The chapter of sensory neuron pharmacology was lacking for some years ago from the pharmacology textbooks. The investigations performed during the last half century led to significant results in this field and the goal seems to be delineated and how to influence the function of sensory neurons. We are not in the posession of a compound acting selectively ont he sensory neurons as an analgesic, but hopefully it will appeare soon. Worthy to metion, that the function of sensory neurons covers not only the conduction from periphery to the centers (orthodromic conduction), but they have effector function (antidromic conduction) as well and can release sensory neuropeptides performing paracrine (and in some cases endocrine) regulation. This effector function might play a role in the inflammatory processes and in the regulation of the microcirculation. The investigation of this system was boosted enormously by the cloning of the capsaicin/vanilloid receptor by the group of David Julius (Caterina et al., 1997). The first description of receptorial action of capsaicin was described on the basis of structure-activity relationships with pharmacological methods (szolcsányi et al. 1975). The cloning of the receptor proved these experiments decades later. In our days pharmacutical companies (Novartis, SKB, Procter and Gamble etc.) are conducting research intensively to discover compounds acting on the sensory neurons selectively.

The sensory neurons are playing a role in the pathomechanism of some illnesses or the illness itself can influence the function of the sensory neurons. Our experiments are focused to the function of sensory neurons from that point of view. During the onco-chemotherapy the neurotoxic side effect occures very frequently. Similarly the neuropathy as a diabetic complication is a problem for the patient and for the medic as well. In these examples not only the sensory neurons are effected, but the autonomic nervous system is involved as well. We focused our interest to the investigation of the function of sensory neurons in experimentally evoked neuropathies because it held a promise to get interesting results.

## 2.1 Experimental sensory neuropathies

Many diseases, medical treatment, drugs may induce neuropathy. Neuropathy is a demonstrable disorder, which clinical symptoms are divided into sensory, autonomic, and sensory-motor neuropathies. In our studies we examined the effect of the changes in sensory-effector functions in experimental conditions. We used experimental models, from their

results may be drawn direct clinical conclusions. The widely used cisplatin induces sensory neuropathy. Diabetic sensory neuropathy is a most common complication of diabetes mellitus. These were the reasons, why we used for experimenthal studies the cisplatin- and diabetes induced sensory neuropathies.

## 2.2 Discovery, effect, use and side-effect of cisplatin

The platinum electrodes produced inhibition of E. coli proliferation. Many platinum-containing compounds were synthetised, but the most active of these subtances were cis-Diamminedichloro-platinum (II) (cisplatin)

Cisplatin is a chemotherapeutic agent used for the treatment of several types of cancer. Unfortunately, cisplatin's therapeutic potential is limited by diverse adverse effects such as myelosuppression, nephrotoxicity, ototoxicity and neurotoxicity The drug-induced neurotoxicity is characterized by a decrease in sensory nerve conduction velocity. Neuromorphologic studies by Barajon cisplatin induced changes revealed an accumulation of sensory neuropeptides calcitonin gene-releted protein (CGRP), substance P (SP), and somatostatin (SOM) in dorsal root ganglia (DRG) with much more severe histological alterations in ganglionic cells than those seen in peripheral fibres. These studies also suggested an impaired axonal transport of sensory neuropeptides by cisplatin.

## 2.3 Connection between asthma and diabetes

Insulin dependent diabetes mellitus, results from an insulin insuffiency that when untreated leads to hyperglycemia, polydipsia, polyuria and weight loss. Streptozotocin (STZ)-induced diabetes in rats produces a condition similar to the clinical form of diabetes mellitus. In addition, STZ-induced diabetes results chronic pain, increasing in vascular permeability and inflammation. Type I diabetes is associated with a low incidence of asthma. The proposal that epithelial damage determines bronchial hyperreactivity presupposes a central role of vagal reflexes supported by a range of findings. Manifestations of hyperreactivity in vivo are not paralleled by altered sensitivity to spasmogens when strips or rings of isolated airway smooth muscle from hyperreactive animals are studied in vitro Furthermore, some forms of hyperreactivity can be prevented by sectioning of the vagus nerve. Considering that almost 90% of the vagal nerve comprises sensory fibers, it is not surprising that capsaicin pretreatment can prevent some forms of airway hyperreactivity. Alternatively, infusion of

sensory neuropeptides induces hyperreactivity in guinea pigs. We have found that the release of sensory neuropeptides, such as that of CGRP, SP and SOM, is significantly decreased from isolated tracheae of rats with diabetic sensory neuropathy. Given this decrease in sensory neuropeptide release together with the welldocumented attenuation of contractile responses of tracheal preparations from insulin-deficient rats to field stimulation (FS) in other studies, we sought to find whether there would be an association between the two processes in the same set of experiments.

## 2.4 Common features of cisplatin or diabetes induced sensory neuropathy

Diabetes induced hyperglycemia overstimulates the polyol pathway and consequenly increases sorbitol formation. Since sorbitol poorly penetrates the cell membrane, its accumulation has been suggested to favor osmotic stress and the development of morphofunctional abnormalities in nerve, lens, etc. The mRNA levels of preproCGRP, preproSP an a preproSOM are significantly reduced in the lumbar 4-6 DRG of STZ induced diabetic male rats compared to the untreated controls. The released amount of the sensorial neuropeptides from axon terminals is decreased both in cisplatin and diabetes induced sensorial neuropathy. So that was the reason for studiing the changes in sensoro-effector function in the above mentioned modells.

## 2.5 Applied methodes in experimental sensory neuropathy

Cisplatin or diabetes induced changes in sensoro-effector function can be studied easiest in isolated bronchial preparation. Bronchial tissue is densely innervated by unmyelinated sensory fibers containing SP, CGRP and
SOM. These sensory nerve terminals locate in bronchial mucosa superficially enough to release neurotransmitters in response to electrical FS at parameters selective for neural elements in sufficient quantities both to be detectable by radioimmunoassay (RIA) and measurement of NANC broncho-constrictory responses. In broncial tissue are densely expressed the genes alpha and betha CGRP receptor (CGRPR), neurokinin-1 ($NK_1$), neurokinin-2 ($NK_2$), and the 4th –type of SOM receptor (SSTR4). The mRNAs of the above mentioned neuropeptide receptors can be easily isolated, and the expression pattern of the genes can be examined by quantitative real time-polymerase chain reaction (QRT-PCR)

# 3 Aim

1. Is the FS induced bronchomotility influenced by sensory neuropathy induced by cisplatin treatment?
    a. Is the FS induced release of sensory neuropeptides from trachea influenced by cisplatin treatment?
    b. Is the plasma levels of neuropeptides (vasoactive intestinal protein (VIP), SP, CGRP, SOM) influenced by cisplatin treatment?
    c. Is the transcription level of the mRNAs of neuropeptide receptors ($NK_1$, $NK_2$, CGRPR, SSTR4) influenced by cisplatin treatment?
2. Is there any relation between the attenuation of field stimulation-induced contractions of the bronchial rings from diabetic rats and the a deficient release of sensory neuropeptide from tracheae in diabetic rats?
    a. Is the plasma SOM level influenced by STZ-idnuced diabetes?
    b. Are FS induced bronchoconstrictions changed by exogene sensory neuropeptides?
    c. Is the antigene induced bronchoconstriction changed in diabetes?

# 4 Methodes

## 4.1 Ethics

The experiments performed in the present work conform to European Community guiding principles for the care and use of laboratory animals. The experimental protocol applied has been approved by the local ethical boards of the Medical Universities of Pécs and Debrecen, Hungary.

## 4.2 Drugs and medicals

| Drugs and Medicals | Producer | Head office of the company |
|---|---|---|
| [Tyr1]- szomatosztatint | Bachem | Budapest |
| 125-Ival jelzett tracerek | Farmakológiai Int. | Pécs, PTE |
| Atropin | Sigma-Aldrich KFT. | Budapest |
| B2M, CGRPR, NK1, NK2, SSTR4 Primerek | Sigma-Aldrich KFT. | Budapest |
| Capsaicin | Fluka | Buchs |
| CGRP | Sigma-Aldrich KFT. | Budapest |
| CGRP, szom antisera | Dr. Görcs T. ajándéka | SOTE, Budapest |
| Cisplatin | TEVA- Biogal | Debrecen |
| EDTA | Sigma-Aldrich KFT. | Budapest |
| Guanetidine | Sigma-Aldrich KFT. | Budapest |
| Inzulin RIA KIT | Izinta KFT | Budapest |
| Inzulin inplantatum | Linplant | Dánia |
| Ovalbumin | Sigma-Aldrich KFT. | Budapest |
| LightCycler RNA Master SYBR Green I Kit | Rosche Applied Science | Budapest |
| L-NAME | Sigma-Aldrich KFT. | Budapest |
| Methanol | Carlo Erba | Limito |
| Na2HPO4 | Reanal | Budapest |
| NaCl | Fluka | Budapest |

| | | |
|---|---|---|
| Piperin | Fluka | Budapest |
| Polipropilen tubes (RIA) | Merck | Darmstadt |
| Rneasy Mini Kit (Quiagene Inc.) | Kasztel Med. KFT | Budapest |
| SP | Sigma-Aldrich KFT. | Budapest |
| SP antisera | Prof. G.J. Dockray ajándéka | |
| SP-tracer | Bachem | Budapest |
| STZ (Zanosar) | Upjohn | Kalamazoo |
| Trasylol | Bayer | Budapest |
| TTX | Sigma-Aldrich KFT. | Budapest |
| Tween 80 | Reanal | Budapest |
| Tyr-α-CGRP | Bachem | Budapest |

## 4.3 Instruments

| Instuments | Producer | Head office of the company |
|---|---|---|
| CWB – 20 water thermostat with circulatory pump | Experimetria Kft. | Budapest |
| TSZ – 04 multi chamber modular tissue bath system | Experimetria Kft. | Budapest |
| CRS – SG bridge amplifier for force measurement | Experimetria Kft. | Budapest |
| Accu Check | Roche- Diagnostic | Budapest |
| LightCycler 1.5r | Roche (Magyarország) Kft. | Budapest |
| Nanodrop | BIO-SCIENCE Ltd. | Budapest |

## 4.4 Nerve conduction velocity studies

This series of experiments was carried out to verify/exclude sensory neuropathy. Left saphenous nerve conduction velocity was determined in animals from both groups. Stimulation intensity suprathreshold for „A" fibres was 0,5 V, 5 Hz, and for „C" fibres was 3 V, 5Hz.

## 4.5 Isometric tension measurements

Isolated segments of the main bronchi (2 mm) were mounted horizontally on two small L-shaped glass hooks of which one was connected to a force transducer for measurement of isometric tension. Neural effects on contractile activity of the segments were studied by means of FS (100 stimuli at 20 V, 0.1 ms and 20 Hz at an initial tension of 12 mN). To study whether the field stimulation protocol applied was selective for nerve-mediated responses, some rings underwent a period of 10-min pre-incubation with tetrodotoxin (TTX), a fast sodium channel blocker. Stimulation with these parameters failed to elicit any contractile response in the presence of 1 μM TTX.

## 4.6 The changes in transcription pattern of the mRNA of the neuropeptide receptors

The excised trachea and the main bronchi were immediately placed in RNA Later buffer (Qiagen, Inc.) and
kept at -70ºC till processing. Total RNA was isolated with the RNA isolation kit according to the manufacturer
protocol. Primers were designed by the Primer 3 online program.
The primers were:
NK1 forward: tgggcaacgtagtggtgata, reverse: cacggctgtcatggagtaga;
NK2 forward: ggagagtcaaccggtgtcat, reverse: ccgagcaccattctgttttt;
CGRP receptor forward: agaacttgaacgccatcacc, reverse: ggatctcaacagcggtcatt;
SSTR4 forward: gccactgtcaaccatgtgtc, reverse: tcttcctcagcacctccagt;
Beta 2 microglobulin forward: acttcctcaactgctacg, reverse: tggtgtgctcattgctat.

## 4.7 QRT-PCR

QRT-PCR was performed by the SybrGreenI detectionmethod. To compare the different mRNA transcription levels, $C_T$ values were compared directly. $C_T$ is defined as the number of cycles needed for the fluorescence signal to reach a specific threshold level of detection, and is inversely correlated with the amount of specific template nucleic acid present in the reaction. Beta 2 microglobulin (b2m) gene was used as internal control. Only those reactions

were included in the quantitative analysis, which gave a well-defined amplification product both by melting curve analysis and agarose gelelectrophoresis.

### 4.8 Relative quantification of the examined genes ($2^{-\Delta\Delta C}{}_T$)

We compared the RNA transcription of the examined neuropeptide receptor genes with b2m. $\Delta C_T$ was first calculated between the $C_T$ values at the 16th and 22nd day from samples from cisplatin-treated and control animals. In the second step, we subtracted the changes in RNA transcription in samples from control animals from the changes in samples from cisplatin-treated animals to obtain the $\Delta\Delta C_T$. This indicated changes in RNA transcription caused by cisplatin treatment between the 16th and 22nd day normalized to RNA transcription changes in the control samples. A high $\Delta\Delta C_T$ value, if negative or positive, indicated significant changes in the RNA transcription level of the tested gene. A positive $\Delta\Delta C_T$ value indicated down-regulation of RNA transcription, whereas a negative $\Delta\Delta C_T$ indicated an up-regulation of the gene's transcription following cisplatin treatment.

$$\Delta\Delta C_{T11day} = (C_{Treceptor}\text{-}C_{Tb2m})_{11day} - (C_{Treceptor} - CT_{b2m})_{control}$$
$$\Delta\Delta C_{T22day} = (C_{Treceptor}\text{-}C_{Tb2m})_{22day} - (C_{Treceptor} - CT_{b2m})_{control}$$

For normalizing the given data we used the $2^{-\Delta\Delta C}{}_t$ method.

### 4.9 RIA measurements

The total amount of sensory neuropeptides (CGRP, SP and SOM) released by FS from rat tracheae and the changes of the levels of the sensory neuropeptides (CGRP, SP, VIP and SOM) in plasma were examined by RIA, developed in our laboratory.

### 4.10 Blood glucose and insulin levels

Blood glucose level was determined by means of the glucose oxidase method. Peripheral insulin sensitivity and β-cell function was also determined using homeostasis model assessment HOMA-IR = (FPI X FPG)/22.5

and in fasted animals HOMA-%B = (20 X FPI) / (FPG-3.5) (16 h period of fasting preceding sampling) as previously decribing. FPG: fasting plasma glucose concentration (mmol/l); FPI: fasting plasma insulin concentration (μU/ml).

### 4.11 Statistical analysis

The isometric tension and nerve conduction velocity data expressed as means ±standard deviation (S.D.) were evaluated with analysis of variance (ANOVA) followed by a modified t-test according to Bonferroni's method. The data of the changes in FS induced sensory neuropeptide release were evaluated by Student's t-test for unpaired data. The blood chemistry data in cisplatin induced sensory neuropathy obtained from RIA measurements are expressed as means of ± standard error of the mean (SEM) and analyzed by ANOVA followed by Student's t-test supplemented with appropriate post hoc evaluation. In case of data normalization failure, the possibilities for further statistics were left by using Man–Whitney's U-test. The blood chemistry data and sensory neuropeptide levels in STZ-induced sensory neuropathy were evaluated by Student's *t*-test for unpaired data, and expressed as means ± S. D.

Changes considered significant at $p \leq 0.05$. Data of QRT-PCR measurements are expressed by means of ± SEM. Changes were significant if the expression level differed more than two times from corresponding control values.

# 5 Experiments

## 5.1 Impaired bronchomotor responses to FS in guinea-pigs with cisplatin – induced neuropathy

### 5.1.1 Experimental protocol

The study was carried out with 16 male guinea-pigs. The animals were random divided into two experimental groups. Control: animals treated with the solvent for cisplatin (1 ml isotonic NaCl) with 75 mg/kg mannitol i.p. once a day over 6 days; Cisplatin-treated: animals treated with 3 mg/kg cisplatin with 75 mg/kg mannitol i.p. once a day over 6 days. The animals in each group were anaesthetized for nerve conduction velocity studies 24 h after the last cisplatin/vehicle dose. Bronchial rings were then prepared from the same animals for isometric tension measurements.

## 5.2 Results

### 5.2.1 Exclusions

Four cisplatin-treated animals had to be excluded from the experiments, two of them as cisplatin failed to produce any decrease in nerve conduction velocity in either A or C fibres, one because of respiratory insufficiency due to pneumonia and one because of the development of extended skin lesions.

### 5.2.2 NCV

At a stimulation intensity supratreshold applied for A and C fibres conduction velocity significantly decreased in cisplatin – treated animals.

### 5.2.3 Changes in isometric tension in response to FS

Field stimulation in tracheal rings from „control" animals evoked a biphasic response, the first contractile component of which comprised an initial fast and a subsequent slow reaction. This two-phase contraction was followed by a relaxation response. The fast contractile component was abolished in Krebs solution containing 4μM guanetidin and 1 μM atropin „NANC

solution", whereas the slow one disappeared in tissues pre-exposed to 100 μM capsaicin. The NANC relaxation was blocked after a 30-min incubation with 30 μM L-NAME. In rings from animals treated with cisplatin, both the amplitude and duration of the field stimulation-induced contractile phase were significantly attenuated. However, the amplitude of the relaxation phase sensitive to L-NAME was augmented. The contractile „spike" left by pre-incubation with capsaicin, however, was similar in preparations from either group. The NANC contractile responses were not influenced by superoxide dismutase in preparations from the control.

## 5.3 Decreased sensory neuropeptide release in isolated bronchi of rats with cisplatin – induced neuropathy

### 5.3.1 Experimental protocol

The study was carried out with 20 Wistar male rats. The animals were randomized into two experimental groups. Control: animals treated with the solvent for cisplatin (1 ml isotonic NaCl) with 75 mg/kg mannitol i.p. once a day over 5 days; Cisplatin-treated: animals treated with 1.5 mg/kg cisplatin with75 mg/kg mannitol i.p. once a day over 5 days. The animals in either group were anaesthetized for femoral NCV studies 11 days after the last cisplatin/vehicle dose. After completion of these studies, the tracheae with the main bronchi were removed. Two-millimeter long segments from the main bronchi were used for isometric tension measurements, whereas the rest of the tissues were utilized for neuropeptide release studies.

### 5.3.2 Results

#### 5.3.2.1 Exclusions

Two cisplatin-treated animals had to be excluded from the experiments, one of them died, and the other did not show any evidence for the development of sensory neuropathy in response to the cisplatin treatment schedule applied.

#### 5.3.2.2 NCV

At a stimulation intensity supratreshold applied for A and C fibres conduction velocity significantly decreased in cisplatin – treated animals.

#### 5.3.2.3 Changes in isometric tension in response to FS

The rings from cisplatin -treated rats responded with attenuated contractions to FS compared to those from solvent-treated animals. Nevertheless, the relaxation response to the field - stimulation protocol applied was of higher amplitude and shorter duration in rings from the cisplatin-treated animals than in controls.

#### 5.3.2.4 FS induced sensory neuropeptide release from rat tracheae

FS released SOM, CGRP and SP from 0.18 ± 0.01; 0.17 ± 0.01 and 0.86 ± 0.02 to 0.59 ± 0.02; 1.77 ± 0.04 és 5.96 ± 0.03 fmol/mg wet tissue weight. This was significantly attenuated to post-stimulation values of 0.36 ± 0.02; 0.45 ± 0.02 és 4.68 ± 0.24 fmol/mg wet tissue weight for SOM, CGRP and SP in animals 11 days after a 5-day treatment period with cisplatin.

### 5.4 Changes in tracheo-bronchial sensory neuropeptide receptor gene expression pattern and in plasma sensory neuropeptides level in rats with cisplatin-induced sensory neuropathy

#### 5.4.1 Experimental protocol

52 male Wistar rats were used throughout the experiment. Twenty-four animals selected for the QRT-PCR and RIA studies were randomized into two experimental groups. Control: animalstreated with the solvent for cisplatin, 1 ml isotonic NaCl with 75 mg/kg mannitol, i.p., once a day over 5 days. The animals in the „cisplatin-treated group” were given 1.5 mg/kg cisplatin with 75 mg/kg mannitol, i.p., once a day over 5 days. Four animals of the Control and Cisplatin group were used for studying of the expression pattern of the neuropeptide

receptors. Total mRNA was isolated 11 and 22 days following cisplatin treatment. Thirty-six animals were randomized for the measurement of the nerve conduction velocity studies.

### 5.4.2 Results

#### 5.4.2.1 NCV

At a stimulation intensity supratreshold applied for A and C fibres conduction velocity significantly decreased in cisplatin – treated animals.

#### 5.4.2.2 Plasma somatostatin, CGRP and substance P levels

Plasma somatostatin immunoreactivity significantly increased 11 and 22 days after cisplatin treatment. Plasma CGRP level exhibited only a transient increase, whereas plasma substance P did not reveal any change.

#### 5.4.2.3 Plasma insulin and glucose level

Both plasma insulin and fasting blood glucose levels remained unaffected by cisplatin treatment. In accordance with these results, the HOMAIR and HOMA-%B did not show any significant difference between the control and cisplatin-treated group.

#### 5.4.2.4 QRT-PCR measurements

On the 16th day of the experiment the expression of the NK1, NK2 and CGRP receptor mRNAs increased 3.22 ± 1.29, 2.78 ± 1.14 and 1.31 ± 0.14 times the control level, respectively. On the 27th day of the experiment the expression level of the NK1, NK2 and CGRP receptor mRNA decreased to 0.69 ± 0.28, 0.91 ± 0.24 and 0.62 ± 0.07 times to the control, respectively (i.e., the difference is non-significant as compared by the 0 day control values). On the other hand, the expression pattern of SSTR4 mRNA is completely different from the above mentioned neuropeptides receptors mRNA expression pattern. We found a significant increase not only at the 16th day, but also at the 27th day as well. The expression

level of the mRNA of the SSTR4 increased 4.41 ± 2.48 times on the 16th day and 7.72 ± 2.66 times on the 27th day.

## 5.5 Feeble bronchomotor responses in diabetic rats in association with decreased sensory neuropeptide release

### 5.5.1 Experimental protocol

The study was carried out with 48 male Wistar rats and 12 male Dunkin-Hartley guinea pigs. The control animals were treated with the solvent for STZ, whereas the rats in the second group were treated with 50 mg/kg STZ ivto make them diabetic. After 4 week, the STZ-treated animals were further randomized into two additional groups, one of which comprised animals that were supplied with continuous-delivery (4 IU/day) subcutaneous insulin implants. This group was referred to as the insulin-supplemented group.

Treatment with capsaicin. Capsaicin was used to elicit a selective functional deterioration of a significant portion of sensory C fibers. Rats constituting subgroups from normal and diabetic animals were given capsaicin/ solvent subcutaneously in the sequence of 10, 30, and 50 mg/kg single daily doses over 3 days on the 8th week of the experimental period. The animals pretreated with capsaicin were used for further studies after a 3-day period of recovery to avoid nonspecific effects of systemic capsaicin administration.

Mechanical responses to ovalbumin in isolated tracheae from sensitized normal and diabetic guinea pigs. Twelve male Dunkin-Hartley guinea pigs were randomized into two groups. The control animals were treated with the solvent for STZ, whereas the second group of animals was treated with a single intraperitoneal injection of 180 mg/kg STZ. Four weeks after STZ and/or solvent injection, the animals were actively sensitized by two intraperitoneal injections of 1 ml/kg 5% (wt/vol) ovalbumin on two consecutive days. The animals were killed after an additional period of 4 weak for isolated trachea experiments.

Eight weeks after treatment with STZ or solvent, the animals were either exsanguinated for in vitro experiments and laboratory determinations or used for nerve conduction velocity studies. Food was withdrawn 12 h before blood sampling for glucose, plasma insulin, and somatostatin measurements. Insulin and somatostatin immunoreactivity were determined by means of RIA. The lower third of the tracheae with the main bronchi was then isolated for isometric tension measurements and neurotransmitter release studies. Six separate animals per group entered the nerve conduction velocity study group.

### 5.5.2 Results

#### 5.5.2.1 NCV

At a stimulation intensity suprathreshold for A or C fibers, conduction velocity significantly decreased in diabetic rats. In the insulinsupplemented animals, conduction velocity for either A or C fibers did not differ from those determined in the control group

#### 5.5.2.2 Effects of experimental diabetes on body weight, blood glucose, plasma insulin, and SOM levels

The normal animals grew steadily over the 8-wk observation period with an average weight gain of 62 ± 4.1 and 58 ± 6.1 g, respectively. The diabetic animals exhibited a marginal weight loss (5.0 ± 2.1 g). The insulin-supplemented rats failed to grow during the first 4 wk. Insulin supplementation from the slow release implants (~4 IU/day) during *weak 4–8* caused a significant increase in body weight to a level approaching that seen in normal animals. In normal, diabetic, and insulin - supplemented animals, fasting blood glucose levels were 4.4 ± 0.6, 17.4 ± 5.5, and 5.0 ± 0.6 mmol/l ($P \leq 0.001$ between diabetic vs. normal or insulin supplemented), with plasma insulin levels of 11.4 ± 3.2, 2.0 ± 0.4 ($P \leq 0.001$ vs. normal), and 12.9 ± 3.8 μIU/ml, respectively. Fasting plasma somatostatin level significantly increased in diabetic vs. normal animals. In response to insulin supplementation,
plasma somatostatin level renormalized by the end of the 8-wk period. Sampling for these determinations was done at the end of the 8-weak experimental period. The guinea pigs receiving the solvent for STZ exhibited a weight gain of 45 ± 6.4 g over the 8-weak observation period, whereas body weight of the STZ-treated animals did not show any change.

#### 5.5.2.3 Contractile responses to FS

Preparations from normal animals exhibited a biphasic response to FS, i.e., an initial contraction was followed by relaxation. The rings from diabetic rats responded with attenuated monophasic contractions to FS compared with those seen in preparations from

normal or insulin- supplemented animals. In rings from normal rats, both atropine (1 μM) and capsaicin desensitization significantly decreased contractions produced by FS. In addition, an augmented relaxation response was seen after atropine, whereas pretreatment with capsaicin abolished the relaxation response to FS. In preparations from diabetic animals, capsaicin failed to significantly influence contractions by FS. The inhibitory effect of atropine on FS-induced contractions was striking. Atropine revealed a weak FS-induced relaxation response in preparations from diabetic animals. Preparations from the insulin-supplemented animals exhibited essentially similar responses to those seen in preparations from normal rats.

#### 5.5.2.4 Sensory neuropeptide release

FS released SOM, CGRP and SP from 0.17 ± 0.0022, 0.15 ± 0.0022 and 1.65 ± 0.093 to 0.58 ± 0.032, 0.74 ± 0.122 és 5.34 ± 0.0295 in preparations from normal, 0.19 ± 0.016, 0.11 ± 0.019, and 0.98 ± 0.116 to 0.22 ± 0.076, 0.34 ± 0.099, and 1.84 ± 0.316 fmol/mg wet weight in preparations from diabetic rats. Insulin supplementation restored neuropeptide release in rings from STZ-treated rats.

#### 5.5.2.5 Effect of sensory neuropeptides on FS-induced contractions

SOM and CGRP were without effect on isometric tension in mechanically precontracted rings in the absence of FS in preparations from either normal or diabetic animals. SP, however, produced a concentration-dependent increase in tension with maximum contraction of 12.3 ± 2.7 and 13.6 ± 3.4 mN with -log EC50 of 7.1 ± 0.2 and 7.0 ± 0.1 in preparations from normal and diabetic animals, respectively. Therefore, when the effect of SP on FS-induced contractions was studied, the initial tension was reset each time to maintain a 12-mN resting tension before an FS challenge. CGRP (up to 0.1 μM) and SP (up to 1 μM) augmented the contractile response to FS in rings from both normal and diabetic rats. The potentiating effect of either neuropeptide on FS-induced increase in tension was significantly elevated in preparations from diabetic vs. normal animals. SOM decreased contractions by FS in both normal and diabetic preparations with a significantly attenuated inhibitory effect in bronchial rings from diabetic animals.

#### 5.5.2.6 Antigen-induced trachea contraction

In tracheal chains from nondiabetic ovalbumin-sensitized guinea pigs, cumulative increases in ovalbumin concentration in one-log unit steps produced concentration-dependent contractions with maximum values ≈70% of those attained by 1 mM carbachol. The concentration-response curve for ovalbumin, however, was shifted to the right when the tracheal chains were prepared from diabetic animals. The EC50 values for ovalbumin-induced contractions were $4x10^{-10}$ and $6x10^{-9}$ g/ml in chains from normal and diabetic animals, respectively.

The maximum contractions by ovalbumin were also significantly decreased in preparations from diabetic animals.

## 6 Summary

In experimental conditions we examined the changes in sensory effector function of sensory nerves induced by diabetes or cisplatin treatment. The results of the experiments may have direct clinical evidences.

In rings of bronchi from animals suffered from sensory neuropathy FS -induced non – adrenergic, non –cholinergic (NANC) bronchoconstriction was significantly attenuated, however the amplitude of the relaxation phase was significantly reduced in cisplatin – induced sensory neuropathy than in case of diabetic neuropathy.

Cisplatin induces elevated peroxi–nitrit formation. FS induced increase in CGRP, SOM, SP release from tracheae, which was significantly attenuated in preparations from animals with sensory neuropathy. Fasting plasma SOM level was significantly increased in diabetic/cisplatin treated animals. Cisplatin treatment changes the expression pattern of the sstr4, induces significant overexpression in the SSTR4 mRNA.

It is speculated, that enhanced fasting plasma level elevation of SOM evolved desensitization to the effect of somatostatin in rings from diabetic rats, causing a decrease in the bronchial hyperreactivity. The attenuated bronchomotor response is related to a decrease in sensory neuropeptide release in experimental sensory neuropathy, at least in our conditions.

This change in sensoro – effector function and SOM induced decrease in bronchial hyperreactivity may have clinical evidences in treatment of bronchial asthma. Taken the SSTR4 overexpression together with hypersomatostatinaemia it is suggested that cisplatin might be of particular importance as a therapeutic tool in patients with endocrine tumors.

## 7 Acknowledgement

I would like to thanks Prof. Dr. Zoltán Szilvássy, Prof. Dr. János Szolcsányi, Dr. József Németh, Dr. Zoltán Sándor, Dr. Judith Szilvássy, Dr. Zsuzsanna Helyes, Dr. Barna Peitl, Dr. Róbert Porszász.

# 8 List of Publications

Szilvassy, J., Sziklai, I., Racz, T., Horvath, P., Rabloczky, G., Szilvassy, Z., Impaired bronchomotor responses to field stimulation in guinea-pigs with cisplatin-induced neuropathy. Eur. J. Pharmacol (3), 259-65. 2000. IF: 2,432

Szilvassy, J., Sziklai, I., Horvath, P., Szilasi, M., Nemeth, J., Kovacs, P., Szilvassy, Z., Feeble bronchomotor responses in diabetic rats in association with decreased sensory neuropeptide release. Am. J. Physiol Lung. Cell. Mol. Physiol (5) 1023-30. 2002. IF: 4,051

Horvath, P., Szilvassy, J., Nemeth, J., Peitl, B., Szilasi, M., Szilvassy, Z., Decrease sensorial neuropeptid release in isoleted bronchi of rats. Eur. J. Pharmacol. (1-3) 247-52. 2005.
IF: 2,432

Horváth P., Szilvássy Z., Peitl B., Szilvássy J.,Helyes Zs., Szolcsányi J., Németh, J., Changes in tracheo-bronchial sensory neuropeptide receptor gene expression pattern in rats with cisplatin-induced sensory neuropathy Neuropeptides. (1) 77-83. 2006. 2,494 IF: 2,494

Sandor, Z., Varga, A., Horvath, P., Nagy, B., Szolcsanyi, J., Construction of a stable cell line uniformly expressing the rat TRPV1 receptor. Cell Mol Biol Lett. (3) 499-514. 2005.
IF: 0,495

Sari, R., Nemeth, J., Porszasz, R., Horvath, P., Blasig, I., E.,, Ferdinandy, P., Nagy, I., Lonovics, J., Szilvassy, Z., Impairment by lovastatin of neural relaxation of the rabbit sphincter of Oddi. Eur J Pharmacol. (1) 91-7. 2001.
IF: 2,432

# 9 Publications on which this thesis is based on

Szilvassy, J., Sziklai, I., Racz, T., Horvath, P., Rabloczky, G., Szilvassy, Z., Impaired bronchomotor responses to field stimulation in guinea-pigs with cisplatin-induced neuropathy. Eur. J. Pharmacol (3), 259-65. 2000. IF: 2,432

Szilvassy, J., Sziklai, I., Horvath, P., Szilasi, M., Nemeth, J., Kovacs, P., Szilvassy, Z., Feeble bronchomotor responses in diabetic rats in association with decreased sensory neuropeptide release. Am. J. Physiol Lung. Cell. Mol. Physiol (5) 1023-30. 2002. IF: 4,051

Horvath, P., Szilvassy, J., Nemeth, J., Peitl, B., Szilasi, M., Szilvassy, Z., Decrease sensorial neuropeptid release in isoleted bronchi of rats. Eur. J. Pharmacol. (1-3) 247-52. 2005.
IF: 2,432

Horváth P., Szilvássy Z., Peitl B., Szilvássy J.,Helyes Zs., Szolcsányi J., Németh, J., Changes in tracheo-bronchial sensory neuropeptide receptor gene expression pattern in rats with cisplatin-induced sensory neuropathy Neuropeptides. (1) 77-83. 2006. 2,494 IF: 2,494