Calcitonin gene-related peptide expression altered in pulmonary neuroendocrine cells in an experimental model of uraemia

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The undertaken studies is aimed at immunohistochemical localisation, quantitative assessment and functional evaluation of neuroendocrine cells in the lungs of rats with experimentally induced uraemia. Lung and trachea fragments were collected after 1, 2 and 4 weeks from nephrectomy. Paraffin-embedded sections were stained with haematoxylin and eosin and by silver impregnation. An immunohistochemical reaction was then performed with the use of a specific antibody against calcitonin-gene related peptide (CGRP) to identify neuroendocrine cells. Obtained results of the performed studies demonstrated a significantly increased number of CGRP-immunopositive cells in the lungs of applied uraemic rats (4.47±0.97, 7.62±1.61 and 5.72±2.5 neuroendocrine cells/mm^2 of lung section after the 1st, the 2nd and the 4th week, respectively), when compared with that in the control (1.22±0.47 neuroendocrine cells/mm^2 of lung section). The obtained results may be approached as a morphological expression of neuroendocrine cells hyperfunction in the lungs in result of disturbed internal body environment caused by renal parenchyma impairment. The enhanced activity of neuroendocrine cells, observed in the lungs of uraemic rats, was confirmed by results of studies of morphometric parameters, such as: area, diameter, length, width and the circularity index.

Key words: APUD cells, lung, chronic renal failure, rat.

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Pearse's theory, concerning the APUD (Amine Precursor Uptake and Decarboxylation) system has backed up the theoretical considerations on the role of peripheral neuroendocrine (NE) cells in the mechanisms of homeostasis control and protection (Pearse, 1969; Raikhlin and Kvetnoi, 1975). Recent date on identification of the same and similar physiologically active substances, acting within the nervous system as neurotransmitters and neurohormones; and, locally or distantly as hormones within the endocrine system, enables both system to be incorporated into the universal diffuse neuroendocrine system – DNES (Kvetnoi and Ingel, 2000). Functionally, DNES cells are receptor-secretory cells, with their receptors located on the surface of the cellular membrane, the cells reacting by secretion to certain stimuli. The receptors of endocrine cells are capable of receiving chemical stimuli from blood or tissues (Marchevsky and Kleinerman, 1982). The primary target of hormonal action (paracrine effect) is the direct environment of NE cells, first of all, the endothelium and the muscular coat of blood vessels, nerve fibres, and cells of the connective tissue (Fiks and Slodkowska, 1989). Following the classical endocrine theory, once the hormones permeate into blood vessels, they head towards distant target organs, i.e. the more distant object of hormonal effects (Ito, 1999; Tomikawa *et al.,* 1998).

The pulmonary neuroendocrine system is represented in the bronchial-bronchiolar-alveolar airways by single NE cells, as well as by innervated endothelial cell clusters of different cell numbers, identified, for the first time by Lauweryns and Van Lommel (Lauweryns and Van Ranst, 1988; Van Lommel *et al.,* 1999) in the lungs of a human neonate, who have defined them as *neuroepithelial bodies* (NEBs). A cell cluster, which forms NEB, consists of – at least – three cells, reached by non-myelin axons from parabronchial and parabronchi-

olar nerves (Gould *et al.,* 1983; Lauweryns and Van Lommel, 1983). In the ultrastructural picture, the NEB-forming cells, similarly as single NE cells, reveal the presence of characteristic secretory granules. Neither the functions of these two cellular components nor their mutual effects have clearly been understood so far in physiological as well as in pathological conditions.

The location of the pulmonary neuroendocrine (PNE) cells within the airway epithelium and their sensitivity to environmental changes provide them with an outstanding position to receive various signals from the paracellular microenvironment. The received signals induce different biological effects in the cells, including proliferation, motion, differentiation, secretion and many other metabolic processes (Cutz and Jackson, 1999; Ito, 1999).

PNE cells themselves, as well as the PNE cells - secreted peptides, also control the airway and vascular tones during respiration. The calcitonin-gene-related peptide (CGRP), one of many neuropeptides secreted by PNE cells, is a known vasodilating and bronchostenosing agent (Gould *et al.,* 1983; Peake *et al.,* 2000).

No direct causes of the clinical symptoms of chronic renal failure and of its final stage - uraemia - have either been traced or recognised and explained. It is beyond argument that the majority of symptoms result from metabolic disorders, caused by an accumulation of toxic, cellular metabolism-disturbing substances in systemic fluids (Dee *et al.,* 2002). The final stage of CRF is chronic uraemia, characterised by severe metabolic disorders, inducing clinical symptoms in the majority of organs (Adachi *et al.,* 1998).

The most often observed pulmonary complication is pulmonary oedema with pleural exudate, fairly often with an asymptomatic course. The pulmonary oedema results from an increased hydrostatic blood pressure in capillary vessels, the pressure rise being in proportion to fluid overload, as well as to increased permeability of pulmonary capillaries (Kalender *et al.,* 2002; Slomian *et al.,* 2000).

Mechanic and haemodynamic changes in the lungs may occur without any basic clinical syndromes, leading to serious pulmonary function disturbances, which may further result in injuries of the walls of perialveolar capillaries, reducing gas exchange efficiency (Kalender *et al.,* 2002).

Since the spectrum of biological effects, exerted by peptide hormones and biogenic amines, is exceptionally broad, including the key aspects of renal insufficiency mechanisms, such as disturbed electrolyte metabolism, general metabolic disorders with an accumulation of toxic compounds in the system, arterial hypertension, circulatory insufficiency, increased endothelial permeability of pulmonary capillary vessels and many other, it seems of prime importance to study the biology and functional morphology of DNES cells in renal failure.

It may theoretically be assumed that disturbances in electrolyte and hormonal metabolism, observed in chronic renal failure, may doubly affect the functions of NE cells in the respiratory system, leading either to dys- or hyperfunction of DNES cells.

In order to increase the knowledge on the pathogenesis of respiratory disorders in CRF, as well as with regards to the lack of reports on the actual behaviour of NE cells in the respiratory tract, it seemed fairly interesting to attempt a thorough study of the dynamics of functional morphology changes of the pulmonary NE cells in experimentally induced renal insufficiency.

The studies aimed at the immunohistochemical localisation and a quantitative and functional evaluation of NE cells in the lungs of rats with experimental uraemia.

Serum creatinine and urea levels, measured in rats after unilateral nephrectomy and partial decortication of the other kidney, were to reflect and confirm the changes obtained in experimental animals, in comparison to the values of those parameters in control rats.

Materials and Methods

Experimental model

The study was performed on seventy-one young male Wistar rats, their body weight at the beginning of the experiment within 200-220 g (the mean body weight: 210±10 g). The rats were kept in lighted and ventilated conditions with room temperature and maintained day and night rhythm. The animals had a free access to standard granulated chow and drinking water was available *ad libitum*. All the experiments were performed at the same time of the day.

Procedures involving the animals and their care were conducted in conformity with the institution-

al guidelines that were in compliance with national and international law and with guidelines for the use of animals in biomedical research (Giles, 1987).

Study assumptions, aim, schedule and mode of animal treatment were approved by the Senate Committee for Supervision of Experiments on Humans and Animals, Medical University of Bialystok.

The experimental animals were divided into two control groups and one experimental group with rats in which uraemia was induced.

C – a control group: twenty-one rats left intact.

SO – a control group: twenty-one rats submitted to sham operation, i.e., decapsulation and removal of the adherent fat from both kidneys.

U – an uraemic group: twenty-nine rats with experimentally induced uraemia. Since five rats died during the experiment, twenty-four survived rats were submitted to final experiments.

The rats in the control groups and the uraemic animals were divided into the following three subgroups:

1. sectioned after 1 week from the surgery: seven rats from each control group (C1, SO1) and eight animals from the group with experimentally-induced uraemia (M1);

2. sectioned after two weeks from the surgery: seven rats from C2 and SO2 and eight animals from U2;

3. sectioned after four weeks from the surgery: seven rats from C3 and SO3 and eight animals from U3.

Induction of experimental renal insufficiency in rats

Experimental uraemia was induced by the method described by Azzadin (Azzadin *et al.,* 1999) and Ormrod and Miller (Ormrod and Miller, 1980). The rats were anaesthetised by pentobarbital, administered intraperitoneally in a dose of 50 mg/kg. Then, an incision (2-2.5 cm long) was made on the dorsal side, 1 cm from the lumbar spine, at the level of the kidneys, thus obtaining a free approach to the right kidney, followed by its nephrectomy. The approach to the left kidney was obtained in an identical way, followed by removal of 70% of renal cortex, leaving the renal medulla intact. The incised integuments were sutured in layers with catgut.

Histology
Method of experimental material collection and fixation

After 1, 2 and 4 weeks from the surgery, the rats were anaesthetised with pentobarbital and blood was collected from their hearts. Then, the animals were sacrificed by decapitation.

Following thoracotomy, the lungs and the trachea were collected in whole. Immediately after the preparation of the collected organs Bouin's fluid was administered by syringe into the trachea to smooth the pulmonary pleura in the right lung. Following the trachea ligation, the lung was fixed in Bouin's fluid for 24 h in temperature of +4°C. Then, following lobotomy from circumference to the hilus, the anterior part of the superior pulmonary lobe, together with the lower section of the trachea, directly above the tracheal bifurcation, were routinely placed in paraffin blocks and then sectioned by a Leica 2025 rotating microtome.

Applied routine histological staining methods

The obtained 5-μm sections were stained with haematoxylin and eosin (H+E) for general histological evaluation and by Grimelius' method, revealing NE cells, following the impregnation of their cytoplasmatic granules with silver salts (Grimelius, 1968).

Identification of DNES cells by immunohistochemical methods

An immunohistochemical reaction to find the calcitonin gene-related peptide (CGRP) in PNE cells was performed on 5-μm paraffin sections, obtained from the trachea and the anterior part of superior pulmonary lobe of the right lung of the studied animals. In those studies, a specific antibody against CGRP was applied (Cat. No C 8198, in 1:8000 dilution, Sigma-Aldrich), purchased at the Sigma-Aldrich, Saint Louis, Missouri 63103, USA. In the immunohistochemical study, the ABC (avidin-biotin peroxidase complex) was used, according to Hsu et al. (Hsu *et al.,* 1981).

Negative control procedures required omission of each stage of the procedure in turn and replacement of the primary anti-CGRP antisera with buffer TRIS 0,05 M pH=7,4. Positive tissue control consisted of the immunostaining sections of rat brain.

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Immunohistochemical reaction procedure

In short: in deparaffined and hydrated (with the use of pure and not denatured alcohol) sections, the activity of endogenous peroxidase was blocked by 3% H_2O_2 for five minutes. After washing with distilled water and 0.05 M TRIS (pH 7.4) for 5-10 minutes, the sections were incubated with the antibody for thirty minutes in a dark-room in room temperature. Then, the sections were three times washed in TRIS buffer. The ABC method was applied, according to the manufacture's protocol, for identification of the immunohistochemical reaction. A secondary, biotinylated antibody (a detection kit: KIT DAKO LSAB (+) or LSAB 2) was used for 15 minutes, then the sections were washed three times in TRIS-HCl and incubated in streptavidin solution. Mayers haematoxylin was used for cellular nuclei staining.

The obtained results of immunohistochemical staining were submitted for evaluation in an Olympus Bx50 microscope. Cells with CGRP expression were searched for and their topography was observed.

Quantitative analysis

CGRP is a specific neuroendocrine marker and, since its expression in rat is high, it is regarded ideal for both identification and quantitative/qualitative analysis of PNE cells.

Immunopositive cells were counted in ten randomly selected microscopic fields, each field of 0.785 mm^2, in magnification of 200x. Three sections of each rat were analysed. The numbers of positively stained cells were presented as mean values per 1 mm^2 of the analysed lung section area.

Technical problems prevented from performing morphometric studies of NE cells in the trachea.

Biochemical evaluation of the blood

Blood was collected from the heart for coagulation. Collected blood samples were left for twenty minutes in room temperature to coagulate. Then, the cylinders with coagulated blood were centrifuged at 3000 RPM for fifteen minutes. In obtained blood serum, urea and creatinine levels were measured in a Backman-CX4 Analyser, using an *Urée cinetique UV 800* of BioMérieux.

The analysis of the preparations and their photographic documentation were performed with an Olympus Bx50 light microscope, with video circuit and a Pentium 120 PC computer with Lucia G (Nikon) software for microscope image analysis.

Statistical analysis

Statistical analysis was based on the variance analysis test. Verification of variance analysis assumptions was performed by Shapiro-Wilk's test (normal distribution assumption) and Barlett's test (assumption of variance homogeneity in studied groups). Bonferroni's test for multiple comparisons was used for the evaluation of differences among particular groups. The analysis was performed, using the SAS ATAT software package.

Results

No significant differences were found between the control groups of rats, regarding the results of performed studies, therefore, for that reason, only the results of studies on sham-operated animals were taken into account.

Increased concentrations of urea and creatinine are one of the indicators of renal insufficiency degree.

A statistical increase of serum creatinine and urea concentrations was demonstrated in all the groups of animals with experimental renal insufficiency, when compared with respective values in the control groups (Table 1).

PNE cells morphology in routine staining methods

In all studied rats, PNE cells recognition was very difficult at the level of light microscope, following H+E staining. It was sometimes possible to see single cells, distinguished among other cells of the respiratory tract epithelium by dark staining of the nucleus, centrally located in light, weakly eosinophylic cytoplasm (Figure 1). Routine studies by means of H+E staining provide merely a rather approximate possibility to distinguish PNE cells and cannot, by any means, be used for the identification of DNES cells.

The number of cells, revealed by Grimelius' method in the respiratory tracts of uraemic rats, was positively higher than in the animals of the control group (Figure 2A and B).

Table 1. Serum concentrations of creatinine and urea in control and uraemic rats (mg/dL).

	Control	1 week	2 week	4 week	* p-value
Creatinine	0.52±0.052	0.63±0.092	0.94±0.058	0.72±0.12	<0.05
Urea	35.33±5.98	52.91±16.39	95.10±11.90	85.58±9.766	<0.05

Location, morphology and immunohistochemical characteristics of NE cells in the trachea and lung structures of control and uraemic rats

Following immunohistochemical reactions, the NE cells were clearly distinguishable in structures of the analysed organs of all the studied rats. The APUD cells, surrounded by other cells of the respiratory tract epithelium, were distinctive by shape variety, from pyramidal to polygonal, oval or columnar.

In the tracheal epithelium of the control animals, single NE cells could only sporadically be observed (Figure 3).

In the lungs of the control rats DNES cells occurred in the respiratory tract as scattered, single or in small groups (most often 2-3 cells only) (Figure 4). The highest PNE cells quantities were observed in larger bronchia, while the smallest PNE cells numbers occurred in alveolar ducts and alveoli.

Higher numbers of cells with positive reaction to the antibodies against CGRP were found in the rats with experimentally induced uraemia than in the controls.

After *one week* of uraemia, a distinct increase in the number of CGRP-immunopositive NE cells was observed. Single NE cells, as well as NEBs, were localised at various levels of the respiratory tract of studied rats. Both in inferior bronchia and bronchioli, there were numerous but small (2-5 cells) clusters of PNE cells (Figure 5). NEBs, with higher numbers of NE cells were only sometimes identified.

The strongest immunoreactivity of PNE cells, both single in small clusters, as well as in large NEBs, was observed after *two weeks* from uraemia induction. Small, several-cell (2-5 cells) clusters of NE cells (Figure 6) were frequently observed. Occasionally, PNE cells formed large, multi-cell NEBs of various shapes (Figure 7). In the microscope image of rat tracheas of rats from the study group, a stronger expression of CGRP was also found, comparing to the control animals and those after one week of uraemia.

In rats after four weeks of uraemia, NE cells were occurring in the respiratory tract, both as single cells and cell clusters, as well as large NEBs (Figure 8).

Computer analysis of CGRP-immunoreactive PNE cells demonstrated a statistically significant

Table 2. A comparative analysis of the numbers of CGRP-immunopositive NE cells in the lungs of control and uraemic rats. (the mean ± standard deviation).

	control	1 week	2 week	4 week	* p-value
Number of cells/mm^2 of lung section	1.22±0.47	4.47±0.97	7.62±1.61	5.72±2.5	<0.0001

* p-value for the analysis of variance.

Table 3. Results of the test for multiple comparisons. Data representation: p-value; 95% confidence interval for the difference of means.

Comparison groups	Means value
control vs. 1 week	< 0.001 (-0.47; -0.27)
control vs. 2 week	< 0.001 (-0.83; -0.64)
control vs. 4 week	< 0.001 (-0.58; -0.38)
1 week vs. 2 week	< 0.001 (-0.47; -0.26)
1 week vs. 4 week	< 0.05 (-0.21; -0.005)
2 week vs. 4 week	< 0.001 (0.16; 0.36)

increase in the number of NE cells in the lungs of rats with experimentally induced renal insufficiency, when compared to the value in the control animals. Quantitative measurements indicated a considerable increase in the number of CGRP-positive NE cells after one week of experiment, while the highest (6-fold) increase of the cells in question was observed after two weeks from the uraemia-inducing surgery. In turn, after four weeks from the surgery, there was a 3-fold increase in the number of CGRP-positive NE cells, when compared to the values in the control rats, see Table 2.

Analysis of selected NE cells morphometric parameters, i.e., the area (A), diameter (D), length (L), width (W) and the circularity index (C) in the lungs of control and studied rats at different time intervals of renal insufficiency

On the basis of computer analysis of CGRP-immunopositive NE cells microscope image, a number of data were obtained, presented in Tables 4 and 5.

The analysis of variance, performed to compare the values of A, D, L, W and C parameters between the control group after one week and two weeks and the study group after four weeks of uraemia, indicated statistically significant differences for all the studied parameters (Table 4).
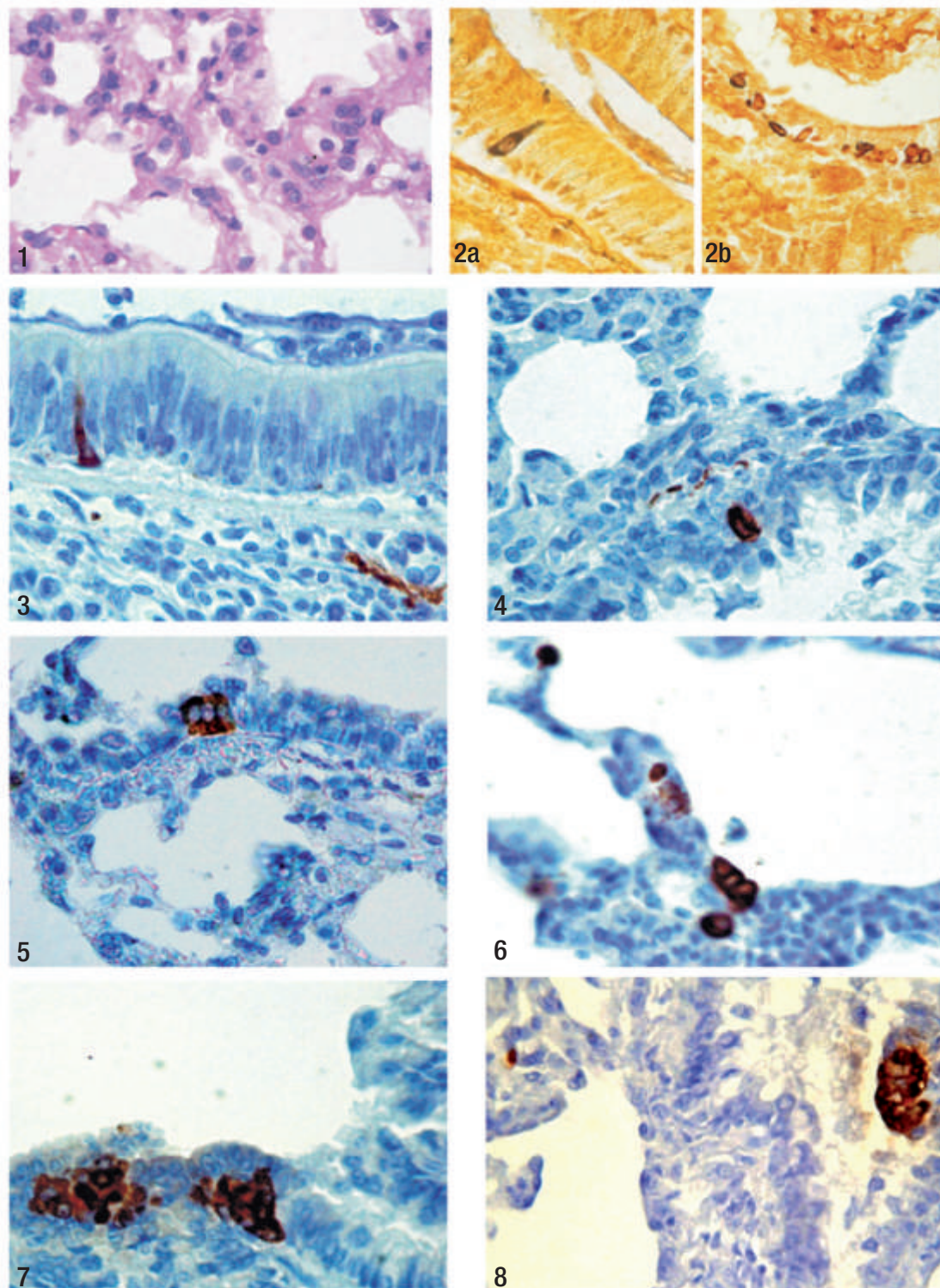
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Figure. 1) A lung fragment of a rat with uraemia after 1 week of the experiment. H+E staining. x250. 2) Lung fragments; a) of a control rat, (in the bronchial epithelium solitary, fusiform neuroendocrine (NE) cell clearly visible), b) of a rat with uraemia after 2 weeks of the experiment (solitary, multiform NE cells visible). Grimelius met. A x400, B x200. 3) In the epithelium of control rat trachea, solitary CGRP-immunopositive NE cell. A fragment of nerve fibre with the studied peptide is visible at the base of the epithelium. x250. 4) Two CGRP-immunopositive NE cells, closely adhering to each other, are visible in the epithelium of control rat bronchus. x250. 5) A small cluster of CGRP-immunopositive NE cells in the epithelium of uraemic rat bronchus after 1 week of experiment. x250. 6) A lung fragment of the rat after 2 weeks of uraemia. An intensive result of immunohistochemical reaction, indicating CGRP in the cytoplasm of NE cells, both single and grouped in small clusters, in epithelium of the respiratory tract. x250. 7) CGRP-immunopositive NE cells, forming large and irregular in shape neuroepithelial bodies in the bronchial epithelium of the rat after 2 weeks of uraemia. x200. 8) A lung fragment from an uraemic rat after 4 weeks of experiment. CGRP-immunopositive NE cells in the neuroepithelial body. x600.

Table 4. Comparative analysis of A, D, L, W and C values. (The mean value ± standard deviation).

	control	1 week	2 week	4 week	* p-value
Area (µm²)	30.89±2.61	27.79±2.19	37.34±1.63	34.87±2.18	<0.0001
Diameter (µm)	6.21±0.25	5.88±0.227	6.83±0.161	6.61±0.218	<0.0001
Length (µm)	6.36±0.36	5.93±0.7	7.09±0.69	7.69±0.68	0.0001
Width (µm)	5.04±0.42	4.83±0.4	5.5±0.34	4.8±0.34	0.0074
Circularity index	0.8±0.03	0.8±0.05	0.79±0.04	0.74±0.03	0.0167

p-value for variance analysis. A-area, D-diameter, L-length, W-width, C-circularity index.

Table 5. Test results for multiple comparisons. (Data representation: *p*-value, 95% confidence interval for the difference of means).

Groups comparison	A	D	L	W	C
control vs. 1 week	> 0.05 (-0.25; 6.45)	> 0.05 (-0.005; 0.66)	> 0.05 (-0.52; 1.4)	> 0.05 (-0.36; 0.79)	> 0.05 (-0.07; 0.05)
control vs. 2 week	<0.001 (-9.8; -3.11)	<0.001 (-0.96; -0.29)	> 0.05 (-1.69; 0.23)	> 0.05 (-1.03; 0.12)	> 0.05 (-0.05; 0.07)
control vs. 3 week	<0.05 (-7.33; -0.64)	< 0.05 (-0.73; -0.06)	< 0.01 (-2.29; -0.36)	> 0.05 (-0.35; 0.81)	> 0.05 (-0.00009; 0.19)
1 week vs. 2 week	< 0.001 (-12.91; -6.21)	< 0.001 (-1.28; -0.62)	< 0.05 (-2.13; -0.2)	< 0.05 (-1.25; -0.09)	> 0.05 (-0.04; 0.076)
1 week vs. 4 week	< 0.001 (-10.43; -3.74)	< 0.001 (-1.05; -0.39)	< 0.001 (-2.7; -0.8)	> 0.05 (-0.56; 0.59)	< 0.05 (0.006; 0.125)
2 week vs. 4 week	>0.05 (-0.88; 5.82)	>0.05 (-0.1; 0.56)	> 0.05 (-1.56; 0.36)	< 0.05 (0.11; 1.26)	> 0.05 (-0.01; 0.108)

A-area, D-diameter, L-length, W-width, C-circularity index.

Discussion

In the course of progressive reduction of the active parenchymal area, clinical symptoms occur in the majority of organs, resulting in severe metabolic disorders. Disturbed pulmonary function and weaker respiration control are among the uraemic consequences in the respiratory tract.

One of the best renal insufficiency indicators is serum creatinine and urea concentration. The significant increase in serum urea and creatinine concentration, obtained during the performed experiment in blood serum of the uraemic rats, comparing to that in the control animals, confirmed the experimentally induced renal insufficiency.

The effects of uraemia on the respiratory tract are still little understood. It is known that pulmonary changes of different character may occur in the course of renal insufficiency (Gibson, 1966; Lee *et al.,* 1975). No factor, directly responsible for their occurrence, has yet been recognised though. The clinical symptoms, observed in renal insufficiency, may, to a certain degree, be connected with PNE cells activity disturbances, even for their high sensitivity to environmental changes and the extremely broad activity spectrum of peptide hormones and biogenic amines, inducing different biological effects.

In the performed experiment, similarly as in the preliminary studies (Azzadin *et al.,* 2001), a distinct increase in the general number of NE cells was observed in different respiratory tract sections of uraemic rats, comparing to the control animals. It was found in a subjective evaluation of cells, stained with silver salts and in the applied immunohistochemical reaction, and confirmed in morphometric studies of NE cells with CGRP expression. Quantitative analysis demonstrated a statistically significant increase in the number of studied cells in all the groups of rats with experimentally induced

uraemia ($p<0.0001$), when compared with the control animals.

The data on the incidence of NE cells in men and in experimental animals are incomplete and, sometimes, very much divergent because of the rare occurrence of PNE cells among cells of the respiratory tract epithelium, as well as because of applications of different measurement techniques.

It appears from the literature review, that the authors take into account various measurement techniques in their studies, concerning the quantitative assessment of pulmonary NE cells (in the predominating majority of cases on the basis of immunohistochemical identification of cells, containing CGRP in their cytoplasm) (IJsselstijn *et al.,* 1998; Yamataka and Puri, 1996). In some reports, the number of PNE cells has been assessed per length unit of the respiratory tract epithelium (Palisano and Kleinerman, 1980; Stevens *et al.,* 1997). Using this approach, some authors have calculated the number of PNE cells/10,000 epithelial cells (Gosney, 1993). In other studies, PNE cells quantity was determined with regards to pulmonary section area (Gillan *et al.,* 1989; Springall *et al.,* 1988).

Since the goal of performed studies was a comparative evaluation of PNE cells in particular groups of studied rats with experimentally induced renal insufficiency, I applied the technique, evaluating the number of CGRP-positive NE cells per 1 mm² of pulmonary section.

The results of quantitative studies of PNE cells, presented in the literature, most often concentrate on lesions of the respiratory tract by various chem-

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ical agents or in result of hypoxia (Peake *et al.,* 2000; Springall *et al.,* 1988; Stevens *et al.,* 1997), while the presented data are, sometimes, contradictory.

It has been proved in experimental conditions that chronic hypoxia unquestionably leads to increasing the numbers of NE cells in the respiratory tract (Pack *et al.,* 1986). Already after four hours of hypoxia did Roncalli *et al.* (Roncalli *et al.,* 1993) observe an increased immunoreactivity of CGRP-like cells in rat lungs, what, according to the authors, resulted from PNE cells secretion disturbances, caused – in turn – by acute hypoxia.

In mine experiment, the increased number of PNE cells, observed in the group of uraemic rats, could be either absolute, i.e., caused by proliferation of those cells, or relative, i.e., resulting from an enhanced synthesis of amines and peptide hormones or from impaired mechanisms of their release. It is to be assumed that those changes were induced by disorders in hormonal and electrolyte metabolism.

The explanation of the increased number of NE cells, observed in renal insufficiency, is rather difficult. An assumption is possible that the increased number of PNE cells is the main, primary pulmonary abnormality, leading to ventilation disorders, however, a much more plausible seems the assumption that the increased number of PNE cells is a secondary event, resulting from earlier disorders of the respiratory tract.

Following the literature data, it is known that numerous cells of the APUD system have – sometimes fairly long – microvilli on the cellular membrane, the microvilli playing the role of receptors. Their presence gives the cells a possibility of reacting to most subtle environmental changes, while by enhancing, decreasing or suppression of their function, associated with hormonal production, they control the activity of a suitable group of cells. It is confirmed by the results of studies by Hoyt *et al.* (Hoyt *et al.,* 1991), indicating an enhanced proliferation of non-endocrine cells, located in a direct vicinity to neoepithelial bodies, what indicates paracrine effects of PNE cells on their close environment.

Perhaps, chronic hypoxia, resulting from pulmonary changes in uraemia, is one of the major factors, inducing the increase of PNE cells number in uraemic rats, as observed in our own studies.

If we assume that the increase in the number of NE cells results from hypoxia, it becomes obvious that hypertrophy must be directly stimulated by pulmonary ventilation disorders (Gillan *et al.,* 1989).

Pathomorphological changes, observed in uraemic patients, may - via different mechanisms - lead to surfactant function disturbances or to injury of the alveolo-capillary barrier, causing a decrease of diffusion capacity for carbon monoxide, what may result in pulmonary oedema (Kalender *et al.,* 2002). In the course of renal insufficiency, frequently observed are pulmonary changes of chronic pulmonary interstitial oedema, what has been confirmed in numerous studies (Slomian *et al.,* 2000).

PNE cells, secreting a number of different neuropeptides, including CGRP, which is a known vasodilator (Stevens *et al.,* 1997) and bronchoconstrictor, are undoubtedly involved in the control processes of respiratory tract tone and blood flow during respiration (Gatto *et al.,* 1989).

The idea and assumptions of the performed own studies are morphological-anatomical in character, rather than physiological, thus the explanation of the pathophysiological mechanism of the NE cell number increase in renal insufficiency may be just a merely hypothetical consideration.

Hypoxy-induced PNE cells hyperplasia seems to be the most probable explanation of the changes, as observed in own studies.

The increase in the general number of PNE cells, observed in own experiment, included an increase in the quantity of both single NE cells, as well as of those clustered into neuroepithelial bodies. On the first and, partially, on the second week of uraemia, the cell clusters were small (less than 5 cells), while on the fourth week, mainly large NEBs were observed.

Such a big increase in the general number of NE cells with CGRP expression, observed in the lungs of rats with experimentally induced uraemia, may reflect the high reactivity of the cells to environmental changes, resulting from renal insufficiency.

The increased activity of PNE cells may be confirmed by the obtained values of morphometric parameters, i.e., the area, diameter, length, width and circularity. The morphometric evaluation of PNE cells demonstrated a slight decrease in values of the above mentioned parameters after one week of uraemia (except the parameter of circularity). It may suggest either activation of the cells, together with their degranulation, or the occurrence – in the

process of hyperplasia – of young, immature forms of NE cells.

It corresponds with the results of studies by Stevens *et al.* (Stevens *et al.,* 1997), who observed PNE cells hyperplasia in mice already after five days from naphthalene administration. The authors suggest that NE cells, when in stimulation conditions, are capable of very fast increase in number.

After two weeks, the values of evaluated parameters in uraemic rats significantly increased, demonstrating the highest values (except the parameter of circularity). The results of morphometric studies of PNE cells in that group of animals may suggest an enhanced biosynthesis of biologically active compounds and of their increased intracellular contents. Also significant changes in PNE cells were observed in the group of studied animals after four weeks from uraemia-inducing surgery. The behaviour of morphological indicators of PNE cells activation in this group of studied cells indicates changes in the shape of cells; perhaps, in result of adaptation processes.

It is possible that the growth of single PNE cells and small NEBs (less than 5 cells) in the uraemic lung is caused by the proliferation and differentiation of NE cells in the respiratory tract epithelium (Peake *et al.,* 2000; Stevens *et al.,* 1997). Some authors suggest differentiation of parent cells of the respiratory tract epithelium in PNE cells and a de novo formation of NEBs (Ito, 1999; Stevens *et al.,* 1997). It is postulated in other studies that PNE cells hyperplasia may result from the proliferation and differentiation of non-endocrine cells.

In uraemia, atrophy of both bronchial and alveolar epithelium (especially of type II pneumocytes) is observed. This phenomenon may be accompanied by regeneration attempts in this epithelium. The proliferation processes of respiratory tract epithelium cells may then be stimulated by PNE cells-secreted mitogenic factors, including CGRP (White *et al.,* 1993). Autostimulation of pulmonary NE cells to proliferation processes in the mechanism of autocrine effects is not unlikely either.

The performed studies have demonstrated that uraemia significantly affects the respiratory system in the organism and the DNES in the lungs. Analysing the obtained data, it should be assumed that the elevated general number of PNE cells and the increased activity of the cells attest their unquestionable role in the modulation of pulmonary function disorders in result of pulmonary lesions caused by renal insufficiency.

Neither the pathophysiological character of these disorders nor the mechanism responsible for PNE cells hyperplasia has yet been unveiled.

Acknowledgments

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