JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2013, 64, 4, 423-428
www.jpp.krakow.pl

Original articles

H. ANTUSHEVICH[1], B. PAWLINA[1], M. KAPICA[2], A. KRAWCZYNSKA[1], A.P. HERMAN[1],
A. KUWAHARA[3], I. KATO[4], R. ZABIELSKI[5]

INFLUENCE OF FUNDECTOMY AND INTRAPERITONEAL OR INTRAGASTRIC ADMINISTRATION OF APELIN ON APOPTOSIS, MITOSIS, AND DNA REPAIR ENZYME OGG1,2 EXPRESSION IN ADULT RATS GASTROINTESTINAL TRACT AND PANCREAS

[1]The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jablonna, Poland;
[2]Department of Biochemistry and Animal Physiology, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, Poland; [3]Laboratory of Physiology, Institute for Environmental Science, University of Shizuoka, Shizuoka, Japan;
[4]Department of Bioorganic Chemistry, Faculty of Pharmaceutical Sciences, Hokuriku University, Kanazawa, Japan;
[5]Department of Physiological Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Warsaw, Poland

Apelin, endogenous ligand of G protein-coupled apelin receptor (APJ), is released into the gastrointestinal lumen, however, local effect of luminal apelin on gut epithelium has not been elucidated so far. The present study aimed to determine the effects of fundectomy, and intraperitoneal or intragastric administration of apelin on pancreatic, gastric and intestinal epithelium apoptosis, mitosis and DNA repair enzyme OGG1,2 expression in adult Wistar rats. Apelin-13 was given by intraperitoneal or gastric gavage twice a day for 10 days (100 nmol/kg b. wt./day). Fundectomized rats did not receive apelin. Control groups received saline as placebo. At the end of the experiment the rats were sacrificed and the pancreas, gastric fundus, duodenum, middle jejunum and colon tissue samples were harvested for immunofluorescence studies. Intraperitoneal and intragastric apelin-13 reduced apoptosis, mitosis and number of DNA damages in rats gastrointestinal tract ($p \leq 0.001$) as compared to control. In fundectomized rats, the apoptotic index in the pancreas and colon was decreased ($p<0.001$), and in the stomach and jejunum was increased ($p<0.001$). Mitotic index was decreased in all gastrointestinal tissues. Number of DNA damages ($p \leq 0.001$) in fundectomized rats was reduced except stomach where OGG1,2 expression was increased ($p \leq 0.001$) as compared to control. In conclusion, circulating and luminal exogenous apelin-13 caused similar effects on intestinal epithelium. Endogenous (gastric) apelin is important for renewal of intestinal epithelium in adult rats. Pharmacological doses of apelin-13 may reduce the cell turnover in the upper gastrointestinal tract epithelium and pancreas, and improve the overall gut health.

Key words: *apelin, fundectomy, apoptosis, mitosis, OGG1,2, DNA damage, cell proliferation*

INTRODUCTION

Apelin was determined to be an endogenous ligand of the orphan G protein-coupled receptor APJ (1). Native peptide of apelin is produced through processing from the C-terminal portion in the pre-proprotein consisting of 77 amino acid residues. During posttranslational processing of pre-proprotein produce several molecular active forms of apelin, consisting of 12, 13, 17, or 36 amino acids and the pyroglutamated apelin-13 (Pyr(1)-apelin-13) (2). A 36-amino-acid variant of apelin appears to be the parent peptide and a 12-amino- acids of apelin is the smallest fragment which able to activate the apelin receptor. These isoforms have distinct activities, with the shorter isoform seeming to be the more potent activator for APJ. Apelin-13 and apelin-36 have different receptor binding affinity and cause different intracellular trafficking of APJ (2, 3). For example the predominant molecular forms of endogenous hypothalamic and plasma apelin in rats were found to be apelin-36, apelin-17, and apelin-13. Pyr(1)-apelin-13 was found to be the predominant isoform in human cardiac tissue. The hydrophobic residues of apelin-13 play important roles in interactions with the APJ receptor (4). The APJ is a typical 380 amino acid 7-transmembrane domain G-coupled receptor showing a close sequence homology to the angiotensin II receptor type 1 (30–40% identity in amino acid sequence), although angiotensin II does not interact with the APJ receptor when expressed in Chinese hamster ovary (CHO) cells or in fibroblasts (1, 5, 6).

Apelin and APJ have a widespread distribution in the body (central nervous system, heart, lung, placenta, mammary gland, adipocytes) (1, 3, 7, 8). In the gastrointestinal tract the expression of APJ receptor was reported in gastric fundic glands, and duodenal tunica mucosa, in particular in the upper half of jejunal villi (9, 10, 11, 12). APJ receptor is expressed in pancreatic acinar, duct and islets cells (9). While apelin peptide was found to be produced chiefly in the stomach mucosa, and to a lesser degree in the duodenum and in jejunum in the villi (9).

Apelin is involved in various physiological functions, such as regulation of homeostasis and food intake (13), stimulates cholecystokinin (CCK) secretion and inhibits the insulin response to intravenous glucose in mice fed high-fat diet (14). Apelin stimulates cardiovascular system development and cardiac muscle contraction, reduction of blood pressure (15, 16) and modulation of the pituitary hormone secretion (13). On cellular level apelin stimulates gastric cells and human vascular smooth muscle cells proliferation (17), promotes angiogenesis (18), suppresses apoptosis in osteoblastic cell line MC3T3-E1 (19), and protects against cardiomyocyte apoptosis (19). However, the effect of apelin on the renewal process of the intestinal mucosa has not been elucidated so far.

The present study aimed to determine the effect of either elimination of endogenous or administration of exogenous apelin. Elimination of a part of circulating apelin pool was achieved by fundectomy. Repeated intragastric or intraperitoneal infusions of apelin were performed to observe the effect of apelin on intestinal epithelium mitosis (expression of Ki67, apoptosis (expression of active caspase-3) and DNA repair (expression of OGG1,2) markers in adult Wistar rats.

MATERIAL AND METHODS

Fundectomized animals

Animal studies have been approved by the Local Ethical Committee. Total, 12 Wistar rats weighing 200 ±10 g were used. Animals were housed in a light and temperature-controlled room with free access to laboratory food and tap water. The night before fundectomy, the rats received a half of their daily food intake. The surgery comprising of partial removal of the gastric fundus was performed in 6 rats under general anaesthesia with atropine sulfate, s.c., 0.15 mg/kg body weight, b. wt, (Polfa, Poland); 2% xylasine, s.c., 3 mg/kg b. wt., (Rometar, PPHU INEX, Poland); and ketamine, s.c., 25 mg/kg b. wt., (Bioketan, Biowet, Poland) supported by local anesthesia with lidocaine, 0.003 mg per stomach (Polfa, Poland) injected into the gastric fundic tissue. A major portion of the gastric fundus was cut off thereby preserving a small portion on the smaller curvature. The remaining stomach was sutured with the two layers of nonabsorbable sutures and laparotomy was closed. The rats from control group (n=6) were "sham operated", it means that animals underwent sham laparotomy involving exposure of the gastrointestinal tissues and gentle palpation for ca. 20 min (20). Antibiotics (Amoxycillin s.c., 150 mg/kg b. wt.; Betamox, Biowet, Poland) were administered intramuscularly after the surgery and repeated after 48 hours. Rats received only little water with glucose on the first postoperative day. After the surgery, rats gradually returned to normal ratios of feed and water within a week and started to gain their body weight. During experiment fundectomized rats did not receive apelin infusions.

Non-operated animals

The animals were housed in a light- and temperature-controlled room with free access to standard food and water. A total of 24 Wistar male rats (200 ±10 g of body weight) were used. During the experiment, the animals were divided into two controls and two experimental groups of 6 rats in each group. Synthetic apelin-13 (Hokuriku University, Japan) was given by gastric gavage or by intraperitoneal injection twice a day for 10 days (100 nmol/kg b. wt./day). Control group received physiological saline as placebo.

At the end of the experiment, the fundectomized and non-operated rats were checked for body weight and sacrificed with barbiturate overdose. For immunofluorescence analyses the following tissues were taken from non-operated rats: pancreas, whole tissue mid-duodenum, mid-jejunum, and colon segments and whole tissue part of gastric fundus. From fundectomized rats a whole tissue part of the stomach nearby surgery line was collected. The tissues were immediately embedded in OCT embedding matrix (Cell Path, UK) and were put into liquid nitrogen. Before further analyses, the frozen tissues were cut into 15 µm cross sections using cryostat (Jung CM 1500, Leica Instruments GmbH, Nussloch, Germany), and were mounted on silanized microscope glass slides (Sigma, USA). The slides were stored in –80°C until immunofluorescence analyses.

Immunofluorescence analyses

For determination of Ki-67 (mitosis marker), active caspase-3 (apoptosis marker), and OGG1,2 (DNA repair marker) expressions, the cross sections were fixed in cold (–20°C) acetone for 10 min, rinsed 3 times in PBS and blocked at room temperature for 1 hour with 1% BSA/PBS (Sigma, USA). For determination of Ki-67 and active caspase-3 expression, the slides were incubated in darkness with, respectively, Ki-67 and Cas-3 antibody (1:500, BD) conjugated with dye fluorescein isothiocyanate (FITC) for 1 hour at room temperature; for determination of OGG1,2 expression the slides were incubated with primary polyclonal goat anti-human and anti-rabbit OGG1,2 antibodies (dilution 1:300, Santa Cruz Biotechnology, Alab, USA). After 1 hour incubation the slides were washed 3 times with PBS, and then incubated in darkness for 1 hour with secondary antibody Rb Anti-Goat IgG with FITC conjugation (dilution 1:50, Calbiochem, USA).

To calculate total cell number, slides were incubated for 10 min in darkness with 7-aminoactinomycin D (7AAD) (1:1000, Sigma, USA). After rinsing 3 times with PBS the labeled sections were covered with immunofluore mounting-medium (Sigma, USA) and covered with cover-glass. Prior visualization, the cells were stored in +4°C in darkness.

Confocal microscopy studies

For semi-quantitative analysis, 10 images (for apoptosis acquired from the apical part of the mucosal membrane, for mitosis and OGG1,2 from the crypt region) were collected by confocal microscope (40× objective, LSM Pascal, ZEISS). Object recognition was performed using the Axio Vision ver. 4.8 Carl Zeiss software. Objects under 50 pixels in size were automatically eliminated and the remaining cells were counted. Quantification was based on the number of cells expressing respective fluorescence compared to the overall number of cells counted on the basis of DNA-related fluorescence.

Statistical analysis

All data were expressed as means ± standard error of mean (S.E.M.). Differences between groups were determined by unparied Student's t-test or nonparametric Mann-Whitney test as appropriate (Statistica v.10.0, StatSoft Poland). In all statistical analyses, $p<0.05$ was taken as the level of significance.

RESULTS

Effect of fundectomy

The apoptotic index *(Table 1*, *Fig. 1 top)* in fundectomized rats was decreased in the pancreas and colon ($p<0.001$) as compared to the control group. However, in the stomach and

jejunum crypts it was increased, and in the duodenum it was unchanged. The mitotic index *(Table 1, Fig. 1 middle)* in fundectomized rats was decreased in all examined gastrointestinal tissues as compared to control group (p<0.001). Enzyme OGG1,2 expression *(Table 1, Fig. 1 bottom)* in fundectomized rats was significantly reduced in all examined gastrointestinal tissues (p<0.001), excepted in the gastric tissue where the OGG1,2 expression was increased (p=0.001) as compared to control.

Effect of exogenous apelin-13

Intraperitoneal administration of apelin-13 caused a significant decrease of apoptotic and mitotic indexes (*Table 2*), in all gastrointestinal tissues as compared to control (p<0.001). Intraperitoneal administrtion of apelin-13 caused a decrease of OGG1,2 expression in the stomach, jejunum and colon (p<0.001) as compared to the control group (*Table 2*). In contrast, in the

Table 1. The effect of fundectomy on the apoptosis, mitosis and OGG1,2 expression in the upper gastrointestinal tract and pancreas of adult rats. Quantification was based on the number of active caspase-3, Ki-67 or OGG1,2 expressing cells compared to the total number of cells counted on the basis of 7-AAD related fluorescence. In the intestine, apoptosis was measured in the villous epithelium. Mitosis and OGG1,2 expression were measured in the crypts. Values are given as %.

		Gastrointestinal tract segments				
	Groups	**Pancreas**	**Fundus stomach**	**Duodenum**	**Mid-jejunum**	**Colon**
Apoptosis	**Control**	37.45 ± 0.93	16.90 ± 0.38	24.13 ± 0.48	20.15 ± 1.20	38.47 ± 0.71
	Fundectomy	14.34 ± 0.26***	20.19 ± 0.66***	25.25 ± 0.84	33.18 ± 0.72***	26.82 ± 0.07***
Mitosis	**Control**	32.08 ± 2.56	17.34 ± 0.85	19.59 ± 0.69	21.17 ± 0.26	7.58 ± 0.27
	Fundectomy	17.18 ± 1.16***	11.29 ± 0.32***	9.48 ± 0.37***	13.53 ± 0.37***	3.88 ± 0.25***
OGG1,2	**Control**	19.94 ± 0.58	11.53 ± 0.34	21.46 ± 0.36	60.23 ± 0.91	36.79 ± 0.92
	Fundectomy	11.55 ± 0.28***	16.60 ± 0.23***	18.08 ± 0.42***	17.48 ± 0.41***	19.03 ± 0.35***

Value are given as means ±S.E.M. (n=6). Unparied Student's t-test or nonparametric Mann-Whitney test (*** p<0.001).

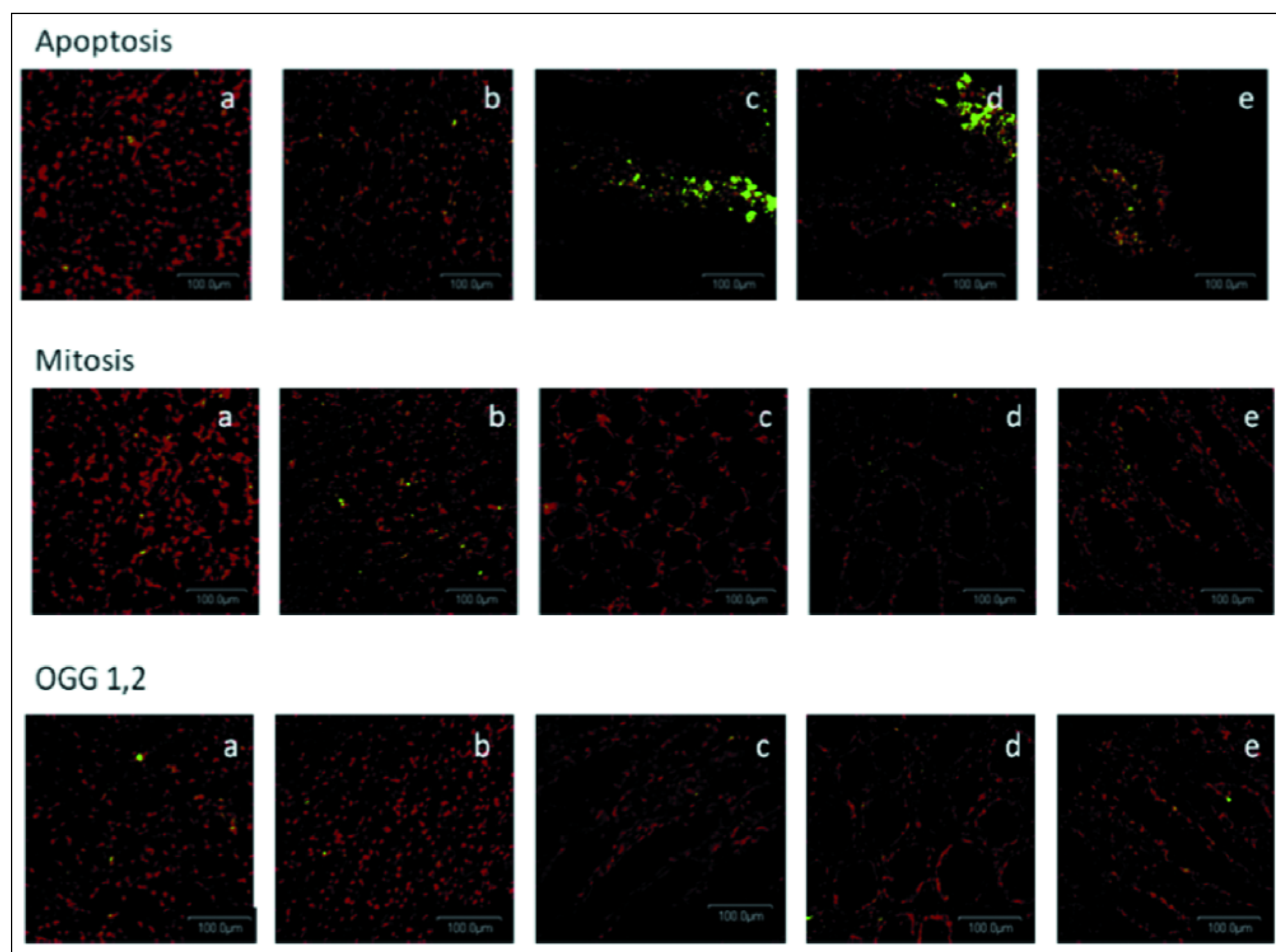

Fig. 1. Representative images from confocal microscope showing apoptosis expression visualized by Cas-3 antibody (*top*), mitosis expression visualized by Ki-67 antibody (*middle*), and expression of DNA repair enzyme OGG1,2 visualized by anti-rabbit OGG1,2 antibody with secondary antibody Rb anti-goat IgG (*bottom*) conjugated with FITC (green fluorescence) in the gastrointestinal tissues of rats after fundectomy (a - pancreas; b - fundus part of stomach; c - duodenum; d - mid-jejunum; e - colon). Red fluorescence visualized cell nuclei stained with 7-aminoactinomycin D (7AAD) (1:1000). Bar =100 µm.

Table 2. The effect of intraperitoneal (i.p.) infusion of apelin-13 (100 nmol/kg b. wt./day for 10 days) on the apoptosis, mitosis and OGG1,2 expression in the upper gastrointestinal tract and pancreas of adult rats. Quantification was based on the number of active caspase-3, Ki-67 or OGG1,2 expressing cells compared to the total number of cells counted on the basis of 7-AAD related fluorescence. In the intestine, apoptosis was measured in the villous epithelium. Mitosis and OGG1,2 expression were measured in the crypts. Values are given as %.

		Gastrointestinal tract segments				
	Groups	**Pancreas**	**Fundus stomach**	**Duodenum**	**Mid-jejunum**	**Colon**
Apoptosis	**Control**	13.45 ± 2.39	12.17 ± 0.98	25.83 ± 1.62	24.69 ± 1.22	25.85 ± 0.34
	Apelin, i.p.	6.49 ± 1.08***	6.19 ± 1.06***	2.39 ± 0.66***	19.58 ± 1.49***	15.62 ± 0.65***
Mitosis	**Control**	18.95 ± 0.39	24.25 ± 0.62	28.68 ± 1.03	25.36 ± 1.47	17.05 ± 1.83
	Apelin, i.p.	4.07 ± 1.02***	4.47 ± 1.29***	9.06 ± 1.22***	11.21 ± 1.16***	8.25 ± 0.59***
OGG1,2	**Control**	6.46 ± 1.07	7.47 ± 1.18	5.74 ± 0.29	6.22 ± 1.17	16.44 ± 0.15
	Apelin, i.p.	8.40 ± 0.95*	2.21 ± 0.91***	5.93 ± 0.83	4.44 ± 0.42***	4.29 ± 0.43***

Value are given as means ±S.E.M. (n=6). Unparied Student's t-test or nonparametric Mann-Whitney test (* $p<0.05$; *** $p<0.001$).

Table 3. The effect of intragastric (i.g.) infusion of apelin-13 (100 nmol/kg b. wt./day for 10 days) on the apoptosis, mitosis and OGG1,2 expression in the upper gastrointestinal tract and pancreas of adult rats. Quantification was based on the number of active caspase-3, Ki-67 or OGG1,2 expressing cells compared to the total number of cells counted on the basis of 7-AAD related fluorescence. In the intestine, apoptosis was measured in the villous epithelium. Mitosis and OGG1,2 expression were measured in the crypts. Values are given as %.

		Gastrointestinal tract segments				
	Groups	**Pancreas**	**Fundus stomach**	**Duodenum**	**Mid-jejunum**	**Colon**
Apoptosis	**Control**	16.25 ± 1.36	10.65 ± 0.74	31.47 ± 0.35	30.73 ± 1.47	26.49 ± 1.67
	Apelin, i.g.	7.65 ± 1.34***	8.40 ± 1.18**	26.14 ± 1.38***	20.52 ± 1.95***	26.60 ± 1.78
Mitosis	**Control**	6.83 ± 0.99	4.87 ± 0.93	4.15 ± 0.33	6.50 ± 0.19	1.97 ± 0.73
	Apelin, i.g.	4.34 ± 0.6***	3.66 ± 0.58*	2.49 ± 0.33***	3.78 ± 0.82***	3.76 ± 0.36***
OGG1,2	**Control**	4.16 ± 0.58	2.25 ± 1.07	6.00 ± 0.68	7.41 ± 0.62	5.03 ± 0.53
	Apelin, i.g.	4.60 ± 0.51	2.82 ± 0.71	3.76 ± 0.66***	2.66 ± 0.62***	3.68 ± 0.5**

Value are given as means ±S.E.M. (n=6). Unparied Student's t-test or nonparametric Mann-Whitney test (* $p<0.05$; ** $p<0.01$; *** $p<0.001$).

pancreas the OGG1,2 expression was increased ($p<0.001$) as compared to control, and unchanged in the duodenum.

Intragastric administration of apelin-13 (*Table 3*, *Fig. 1 bottom*) caused a significant decrease of apoptotic index ($p\leq0.001$) in all, except colon, examined gastrointestinal tissues as compared to control. Intragastric administration of apelin-13 (*Table 3*) significantly reduced the number of mitotic cells in all, except colon, examined gastrointestinal tissues ($p<0.001$) as compared to control group. In the colon, however, the mitotic index was increased ($p<0.001$) as compared to control. Intragastric administration of apelin-13 (*Table 3*) reduced enzyme OGG1,2 expression in the duodenum, jejunum and colon ($p<0.01$) as compared to the control group. No effect of intragastric apelin-13 on OGG1,2 expression in the pancreas and stomach was observed.

DISCUSSION

In brief, our results showed that both exogenous apelin-13 administrations, intraperitoneal and intragastric, markedly reduced cell apoptosis, mitosis and DNA damages in all examined gastrointestinal tissues. Different results were obtained in animals with part of apelin producing cells removed by fundectomy. In fundectomized rats, the number of apoptotic cells in the stomach and jejunum was increased which was in agreement with infusion studies. In contrast, in the pancreas and colon the apoptosis was decreased. Mitotic index in fundectomized rats was decreased in all gastrointestinal tissues examined which contrasts with infusion studies. Regarding OGG1,2 expression, only results from the stomach are in agreement with infusion studies, whereas in the other tissues the results are opposite from expected following infusion studies.

Concerning infusion studies, similar effects were obtained after intragastric and intraperitoneal administration which suggests that endogenous apelin may easily pass into gastric lumen like into the circulation, and thereby act on the mucosa from both, circulating blood and gastrointestinal lumen. However, effect of apelin on pancreatic enzymes activity depends on the way of administration: apelin administered into the duodenal lumen significantly increased pancreatic protein and trypsin outputs and intravenous boluses of apelin reduced the juice volume, protein and trypsin outputs (21). Our injection studies also revealed that exogenous apelin seem to inhibit the renewal of the gastrointestinal tissues, though without disrupting gut epithelium integrity or inducing local inflammatory response (histology data not shown). The differences between infusion and fundectomy studies indicate that physiological apelin of gastric origin may control epithelial cell turnover, at least in the stomach.

In fundectomized animals an increase in the number of DNA damages helps to explain increased apoptosis. Fundectomy reduces luminal apelin and it may in turn increase cell apoptosis in the small intestine, what suggests that endogenous gastric apelin can be involved in small intestinal cell turnover. The discrepancies in results between fundectomized and infused rats suggest that other than apelin regulatory factors may notably control epithelial regeneration. The number of endogenous regulators that stimulate gastrointestinal epithelial cells turnover is high, including

autonomic nerves, leptin, ghrelin, gastrin, CCK, growth factors (*e.g.*, EGF, TGF-α), and many other. Apelin interacting with them, may influence their release into blood, and thereby affect epithelial renewal as well as other physiological function in the pancreas, stomach and small intestine (22, 23). It was previously described that intraperitoneal administration of apelin in normal and obese mice reduced insulin and leptin level (14). In our study, apelin reduced number of cells with DNA damages and suppressed apoptosis and mitosis evidences of its protective effect on the cells. Observed phenomenon, when less cells divide and less cells die, suggests that apelin may improve cell general condition. In our recent *in vitro* study we have confirmed this phenomenon in intestinal crypt cell line IEC-6 (data not published).

The effect of apelin on the mitosis and apoptosis processes is intensively investigated in cardiovascular and in the other tissues, no studies were performed in gastrointestinal tissues so far. Also there are no studies, to our knowledge, on the effects of apelin on DNA repair processes in gastrointestinal tissues. Other reports showed that apelin may either stimulate (19, 24, 25, 26) or inhibit (24, 27) cell proliferation depending on the cell line concerned. Acute exposure of rat pups to hypoxia lowered gastric and colonic epithelial cell proliferation and hypoxia in combination with apelin treatment increased epithelial proliferation (24). In the *in vitro* study, apelin increased cell proliferation of enteric cells exposed to hypoxia (24). Apelin-13 also stimulates gastric, osteoblastic and vascular smooth muscle cells proliferation (17, 19, 25, 26). To explain mechanisms concerned, Li *et al*. (25) reported that apelin-13 decreased the proportion of cell in the G0/G1 phase, while increased the number of cells in S phase and increased the levels of cyclin D1, cyclin E and pERK1/2. In turn, Liu *et al*. (26) suggested that apelin promoted vascular smooth muscle cells (VSMCs) proliferation through PI3K/Akt signaling transduction pathway. Apelin did not increase cell proliferation in human non-small cell lung cancer NSCLC cell *in vitro*, but increased apelin levels by gene transfer to NSCLC cells stimulated tumor growth and microvessel densities and perimeters *in vivo* (27). Kasai *et al*. (28) suggested that apelin suppressed proliferation of endothelial cells independent of vascular endothelial growth factor VEGF/VEGF receptor 2 signaling pathway and that apelin is a prerequisite factor for hypoxia-induced retinal angiogenesis.

While reports concerning the effect of apelin on cell proliferation are conflicting, then these concerning involvement of apelin in cell apoptosis emphasize with one consent its anti-apoptotic function, including our data. Namely, apelin inhibited cardiomyocyte and human vascular smooth muscle cells (VSMCs) apoptosis induced by serum deprivation trough increasing Akt and mTOR phosphorylation (29, 30). Xie *et al*. (31) and Tang *et al*. (19) reported that apelin suppressed apoptosis of human and mouse osteoblasts through a APJ/PI-3 kinase/Akt signaling pathway. Apelin-13 also reduced apoptosis in bone marrow mesenchymal stem cells (BMSCs) from adult rats - the anti-apoptotic effects were blocked by inhibiting MAPK/ERK1/2 and PI3K/Akt signaling pathways (32).

In summary, intraperitoneal and intragastric administrations of apelin-13 causes similar effect on intestinal epithelium mitosis, apoptosis and DNA repair enzyme (OGG1/2) expression. Endogenous gastric apelin contributes to the renewal of the intestinal epithelium in adult rats. Pharmacological doses of apelin-13 may reduce the cell turnover in the upper gastrointestinal tract epithelium and pancreas, and improve the overall gut health.

Acknowledgements: This study was supported by Ministry for Research and Higher Education, Poland. Grant N N311 082737.

Conflict of interests: None declared.

REFERENCES

1. Tatemoto K, Hosoya M, Habata Y, Fujii R, Kakegawa T, Zou M. X, *et al*. Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor. *Biochem Biophys Res Commun* 1998; 251: 471-476.
2. Lee DK, Cheng R, Nguyen T, *et al*. Characterization of apelin, the ligand for the APJ receptor. *J Neurochem* 2000; 74: 34-41.
3. De Mota, Lenkei N, Llorens-Cortes C. Cloning, pharmacological characterization and brain distribution of the rat apelin receptor. *Neuroendocrinology* 2000; 72: 400-407.
4. Higuchi K, Masaki T, Gotoh K, *et al*. Apelin, an APJ receptor ligand, regulates body adiposity and favors the messenger ribonucleic acid expression of uncoupling proteins in mice. *Endocrinology* 2007; 148: 2690-2697.
5. Kawamata Y, Habata Y, Fukusumi S, *et al*. Molecular properties of apelin: tissue distribution and receptor binding. *Biochim Biophys Acta* 2001; 1538: 162-171.
6. O'Dowd BF, Heiber M, Chan A, *et al*. A human gene that shows identity with the gene encoding the angiotensin receptor is located on chromosome 11. *Gene* 1993; 136: 355-360.
7. Foldes G, Horkay F, Szokodi I, *et al*. Molecular and functional characteristics of APJ. Tissue distribution of mRNA and interaction with the endogenous ligand apelin. *J Biol Chem* 2000; 275: 21061-21067.
8. Medhurst AD, Jennings CA, Robbins MJ, *et al*. Pharmacological and immunohistochemical characterization of the APJ receptor and its endogenous ligand apelin. *J Neurochem* 2003; 84: 1162-1172.
9. Kapica M, Jankowska A, Antushevich H, *et al*. The effect of exogenous apelin on the secretion of pancreatic juice in anaesthetized rats. *J Physiol Pharmacol* 2012; 63: 53-60.
10. Susaki E, Wang G, Cao G, Wang HQ, Englander EW, Greeley GH. Apelin cells in the rat stomach. *Regul Pept* 2005; 129: 37-41.
11. Wang G, Kundu R, Han S, *et al*. Ontogeny of apelin and its receptor in the rodent gastrointestinal tract. *Regul Pept* 2009; 158: 32-39.
12. Wang G, Anini Y, Wei W, *et al*. Apelin, a new enteric peptide: localization in the gastrointestinal tract, ontogeny, and stimulation of gastric cell proliferation and of cholecystokinin secretion. *Endocrinology* 2004; 145: 1342-1348.
13. Taheri S, Murphy K, Cohen M, Sujkovic E, Kennedy A, Dhillo W. The effects of centrally administered apelin-13 on food intake, water intake and pituitary hormone release in rats. *Biochem Biophys Res Commun* 2002; 291: 1208-1212.
14. Castan-Laurell I, Dray C, Attane C, Duparc T, Knauf C, Valet P. Apelin, diabetes, and obesity. *Endocrine* 2011; 40: 1-9.
15. Ishida J, Hashimoto T, Hashimoto Y, *et al*. Regulatory roles for APJ, a seven-transmembrane receptor related to angiotensin-type 1 receptor in blood pressure in vivo. *J Biol Chem* 2004; 279: 26274-26279.
16. Tatemoto K, Takayama K, Zou MX, *et al*. The novel peptide apelin lowers blood pressure via a nitric oxide-dependent mechanism. *Regul Pept* 2001; 99: 87-92.
17. Wang G, Anini Y, Wei W, *et al*. Apelin, a new enteric peptide: localization in the gastrointestinal tract, ontogeny, and stimulation of gastric cell proliferation and of cholecystokinin secretion. *Endocrinology* 2004; 145: 1342-1348.
18. Rayalam S, Della-Fera MA, Kasser T, Warren W, Baile CA. Emerging role of apelin as a therapeutic target in cancer: a patent review recent pat anticancer *Drug Discov* 2011; 6: 367-272.

19. Tang SY, Xie H, Yuan LQ, *et al*. Apelin stimulates proliferation and suppresses apoptosis of mouse osteoblastic cell line MC3T3-E1 via JNK and PI3-K/Akt signaling pathways. *Peptides* 2007; 28: 708-718.
20. Matyjek R, Kapica M, Puzio I, Babelewska M, Zabielski R. The effect of fundectomy on pancreatic secretion in anaesthetized rats. *J Physiol Pharmacol* 2004; 55(Suppl. 2): 69-75.
21. Kapica M, Puzio I, Kato I, Kuwahara A, Zabielski R. Role of feed-regulating peptides on pancreatic exocrine secretion. *J Physiol Pharmacol* 2008; 59(Suppl. 2): 145-159.
22. Szczepanska-Sadowska E, Cudnoch-Jedrzejewska A, Ufnal M, Zera T. Brain and cardiovascular diseases: common neurogenic background of cardiovascular, metabolic and inflammatory diseases. *J Physiol Pharmacol* 2010; 61: 509-521.
23. Tarczyk Z, Trzebski A. Fizjologia czlowieka z elementami fizjologii stosowanej i klinicznej. Warszawa, Wydawnictwo Lekarskie PZWL, 2001.
24. Han S, Wang G, Qi X, Lee HM, Englander EW, Greeley GH Jr. A possible role for hypoxia-induced apelin expression in enteric cell proliferation. *Am J Physiol Regul Integr Comp Physiol* 2008; 294: 1832-1839.
25. Li F, Li L, Qin X, *et al*. Apelin-induced vascular smooth muscle cell proliferation: the regulation of cyclin D1. *Front Biosci* 2008; 13: 3786-3792.
26. Liu C, Su T, Li F, *et al*. PI3K/Akt signaling transduction pathway is involved in rat vascular smooth muscle cell proliferation induced by apelin-13. *Acta Biochim Biophys Sin* 2010; 42: 396-402.
27. Berta J, Kenessey I, Dobos J, *et al*. Apelin expression in human non-small cell lung cancer: role in angiogenesis and prognosis. *J Thorac Oncol* 2010; 5: 1120-1129.
28. Kasai A, Ishimaru Y, Kinjo T, *et al*. Apelin is a crucial factor for hypoxia-induced retinal angiogenesis. *Arterioscler Thromb Vasc Biol* 2010; 30: 2182-2187.
29. Cui RR, Mao DA, Yi L, *et al*. Apelin suppresses apoptosis of human vascular smooth muscle cells via APJ/PI3-K/Akt signaling pathways. *Amino Acids* 2010; 39: 1193-1200.
30. Zhang Z, Yu B, Tao GZ. Apelin protects against cardiomyocyte apoptosis induced by glucose deprivation. *Chin Med J* (*Engl*) 2009; 122: 2360-2365.
31. Xie H, Yuan LQ, Luo XH, *et al*. Apelin suppresses apoptosis of human osteoblasts. *Apoptosis* 2007; 12: 247-254.
32. Zeng X, Yu SP, Taylor T, *et al*. Protective effect of apelin on cultured rat bone marrow mesenchymal stem cells against apoptosis. *Stem Cell Res* 2012; 8: 357-367.

Received: May 21, 2013
Accepted: August 15, 2013

Author's address: Hanna Antushevich, The Kielanowski Institute of Animal Physiology and Nutrition of the Polish Academy of Sciences, Laboratory of Molecular Biology, 3 Instytucka Street, 05-110 Jablonna, Poland
E-mail: antuszewicz@op.pl