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Endocrine cells in pig's gallbladder, ductus cysticus and ductus choledochus with special reference to ghrelin

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In human biliary pathways and gallbladder there have been several reports describing endocrine cells (ECs) mainly in chronic inflammation. In pigs' biliary structures we couldn't find data about ECs. Ghrelin is peptide hormone participating in the growth-hormone-release and in modulation of food intake. It has also pro-inflammatory functions. Ghrelin-positive ECs are the main source of Ghrelin. The present study reveals the presence of ghrelin+ ECs in pigs' gallbladder cystic and choledochal duct – by immunohistochemistry. In pigs gallbladder ECs are very rare. Single Chromogranin A^+, Somatostatin$^+$ and Serotinin$^+$ ECs were observed. In choledochal duct there are Chromogranin A^+, Somatostatin$^+$, Gastrin$^+$ and Ghrelin+ECs more in number as compared to gallbladder. Most ECs were located in d.cysticus. They were also Chromogranin A^+, Somatostatin$^+$, Gastrin$^+$ and Ghrelin$^+$ ECs. In conclusion we support that various ECs including Ghrelin exert action on physiology and pathology conditions in biliary tree in pigs.

Key words: endocrine cells, ghrelin, pigs' biliary system

INTRODUCTION

Endocrine cells had been found to be widely distributed in the epithelial structures in gastrointestinal tract [1, 2]**.** In human embryo, the first anlage of the bile ducts and the liver was the hepatic diverticulum or liver bud, that occurred on eighteen day in the anterior intestinal portal [3] and it's endodermal origin was already demonstrated [2]**.** Endocrine cells in human gallbladder and biliary pathways were investigated mainly in disease [4]. In human biliary pathways endocrine cells presence was associated with dysplasia and metaplasia around malignant tumors [5, 6], developmental mistakes and in chronic conditions [7, 8].

Endocrine cells and nerve structures had been investigated in the gastrointestinaltract of different animal species [9-14].There existed some reports on the existence of endocrine cells in the biliary and pancreatic ducts of rabbit, rat, cat and sheep [15, 16, 17]. At least eight immunohistochemically distinct endocrine cell types were described in the bile ducts of some vertebrate species as followed: motilin and substance P (SP) in rabbits [16]; insulin**,** glucagon, somatostatin, pancreatic polypeptide (PP) and cholecystokinin (CCK) in rat common pancreatic bile duct [18]; insulin**,** glucagon, CCK, PP and somatostatin in rat bile duct in diabetes[19]; serotonin and somatostatin in pigs' bile duct and gallbladder [12]; somatostatin in pigs' gallbladder and biliary pathways [12]; insulin**,** glucagon, PP, somatostatin in extrahepatic bile ducts of hilar region in mice [20]. Our previous investigation on endocrine cells in human common bile duct in obstructive jaundice showed chromogranin A (CHA), synaptophysin, somatostatin (SOM), serotonin (SER), gastrin (GAS) and secret inimmunoreactivity (IR) in choledochal endocrine cells and an increase of these cells in chronic inflammation [7].

Ghrelin-immunoreactivity (IR) cells were identified mainly in vertebrate stomach [21, 22, 23]. Ghrelin consists of 28 aminoacids, including O-n- octanoylated Ser^3 residue essential for growth hormone release [21]. Ghrelin's physiological and pathophysiological significance had been extensively studied since its discovery in 1999[24, 25]**.** In rodents ghrelin-producing cells were observed all over the gastrointestinal tract: gastric body, antrum, duodenum, ileum, cecum, colon [26]. Ghrelin IR was described in distinct cells of pancreas, pituitary, lung and thyroid [27-32]. Ghrelin positivity was observed also in some immune cells in human (T cells, B cells and neutrophils), [33]. Ghrelin mRNA was amplified

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from multiple tissues, but for many of these any cellular confirmation is still lacking [34].

To our knowledge there was no data about endocrine cells and about ghrelin-positive endocrine cells in pigs'gallbladder, cystic duct and choledochal duct. The aim of the present study was to describe chromogranin-, somatostatin-, serotonin-, gastrin-and ghrelin-positive endocrine cells in pig's gallbladder, cystic and choledochal ducts.

MATERIALS AND METHODS

Animals and tissues sampling

The material was obtained from the gallbladder's neck, middle parts of ductus cysticus and ductus choledochus of 6 male pigs (Landras X Danube White). The animals aged 6 months, weighing 92-98 kg, slaughtered for meat consumption in a slaughterhouse in accordance with the Bulgarian lows. In the current study cross serial sections of the mentioned organs were used. A total of 6 pigs were used in this study.

Immediately after slaughtering, small specimens from gallbladder'sneck, middle part of ductus choledochusand ductus cysticus where taken for each animal. The samples (total number=18) were promptly fixed in 4% para-formaldehyde in 0.01 M phosphate-buffered saline (PBS), pH 7.4, for 24 h at 4˚C. After that the specimens were dehydrated in graded series of ethanol, cleared with xylene and embedded in paraffin. Microtome sections (4 μm-thick) were cut and collected onto slides treated with poly-L-lysine. One section of each sample was stained with hematoxylin and eosin (HE) and examined under a light microscope (LEICA DM 1000, Germany) to assess the morphology and exclude pathological changes. The other sections were treated by immunohistochemistry.

Immunohistochemistry

Immunohistochemical staining for chromogranin (CHA), gastrin (GAS), somatostatin (SOM), serotonin (SER), ghrelin (GHR), (Table 1), was performed using avidin-biotin-peroxidase complex technique on formalin-fixed and paraffin-embedded tissue sections as described earlier [7]. Paraffin sections 4μm thick were dewaxed in two xylenes at 56°C for 1 h, and were rehydrated in ethanol. Later, they were washed in 0.1 M PBS, pH 7.4, boiled for 20 min at 100°C, cooled at room temperature, incubated in 1.2 % hydrogen peroxide in methanol for 30 min, and rinsed in 0.1 M phosphate buffer, pH 7.4, for 15 min. The sections were then blocked for 30 min with normal mouse/rabbit serum (DAKO). After incubating with the primary mouse/rabbit antibodies overnight, they were washed in PBS, pH 7.4, and incubated with a secondary antimouse/antirabbit biotinylated antibody (DAKO ready-to-use LSAB®2 System, HRP K0675) for 4h, and subsequently with the streptavidin-HRP complex (DAKO ready-to-use LSAB®2 System, HRP K0675) for 4 h. All incubations were performed in a moist chamber. The reaction was made visible by using a mixture of 3 mg 3,3'-diaminobenzidine (DAB) (Sigma, St. Louis MO, USA) in 15 ml 0.05 Tris-HCL buffer, pH 7.5, and 36 μl 1% hydrogene peroxide for 10-20 min, rinsed in distilled water. The sections were dried overnight at room temperature, and then mounted with entelan for light microscopy. They were counterstained with Mayer's hematoxylin.

Sections incubated with non-immune sera instead of the primary antibodies were used as negative controls.

RESULTS

The structure of different swine gallbladders and bile ducts was judged to be fully normal in the different studied samples.

Gallbladder

In pig's gallbladder endocrine cells were very rare. From six different specimens endocrine cells could be observed in only two. Endocrine cells were found mainly in the intramural glands. They were CHA- (Fig. 1), SER-, and SOM- positive.

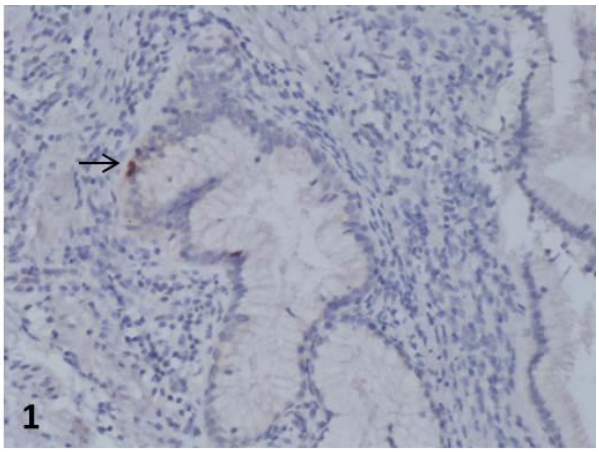

Fig. 1. Chromogranin A positive endocrine cell (arrow) in gallbladder (x 200).

Endocrine cells were located mainly on the basement membrane of the glands and were of closed type.

Ductus choledochus

From all six samples endocrine cells were observed in five of them, in mural glands. The

density of endocrine cells was increased as compared to that in gallbladder. CHA-positive cells were most numerous (Fig.2a).

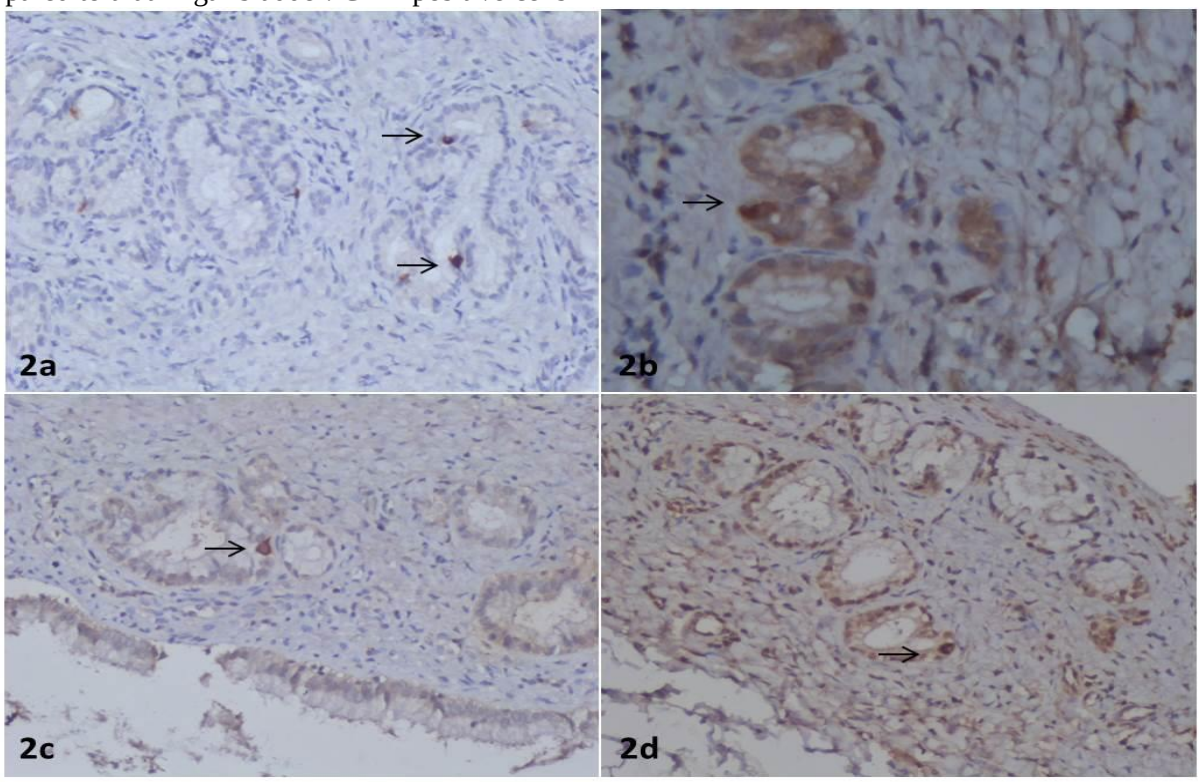

Fig. 2. a) Chromogranin A positive endocrine cells (arrows) in ductus choledochus (x 100); b) Somatostatin positive endocrine cell (arrow) in ductus choledochus (x 200); c) Gastrin positive endocrine cell (arrow) in ductus choledochus (x 200); d) Ghrelin positive endocrine cell (arrow) in ductus choledochus (x 100).

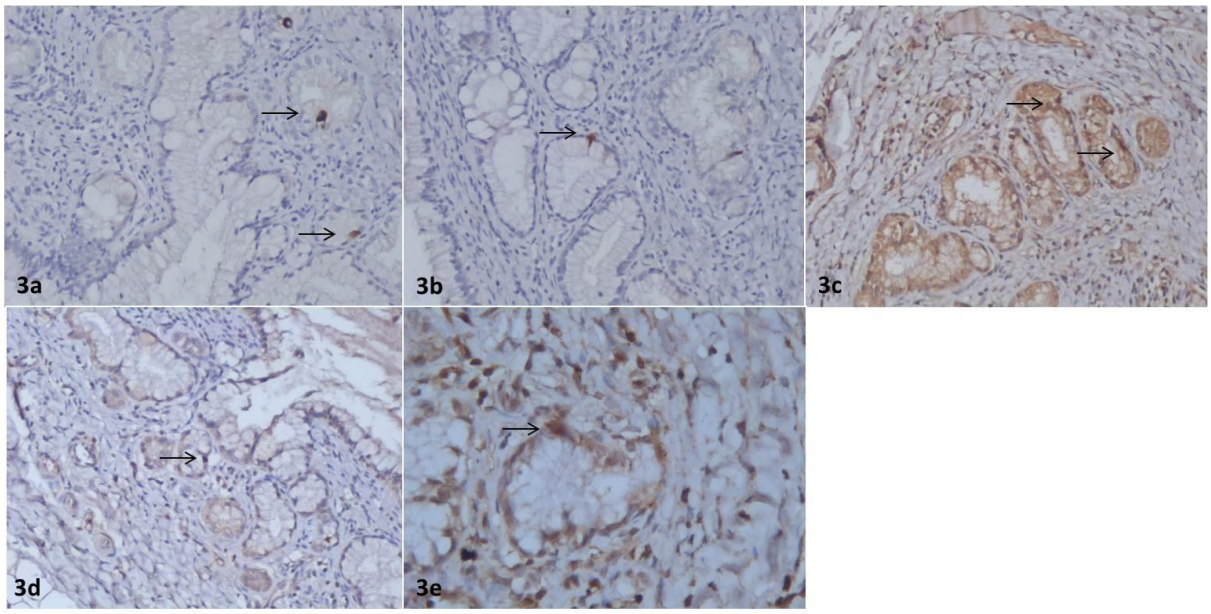

Fig. 3. a,b) Chromogranin A positive endocrine cells (arrows) in ductus cysticus (x 100); c) Somatostatin positive endocrine cells (arrows) in ductus cysticus (x 100); d) Gastrin positive endocrine cell (arrow) in ductus cysticus (x 100); e) Ghrelin positive endocrine cell (arrow) in ductus cysticus (x 200).

SOM immunoreactive (IR) endocrine cells were seen at the base of glands (Fig.2b). In the connective tissue around glands there could be observed SOM-positive peptidergic nerve fibers. SER-positive endocrine cells were also scattered in some glands. Single GAS-positive endocrine cells were located in glands structures beneath the surface epithelium (Fig. 2c).

GHR-positivity was observed in endocrine cells in the intramural glands (Fig. 2d), in some nerve fibers and connective tissue cells (mast cells).

Ductus cysticus

Endocrine cells were observed in all six samples and were more as compared to the other two locations. Of them CHA-positive cells were most numerous. Most of these endocrine cells were in close proximity to goblet cells in the same glands and were from the closed type (Fig. 3a).

Some endocrine cells showed opened type (Fig. 3b). SOM-positive endocrine cells were observed in the glands and surface epithelium (Fig. 3c). GAS IR endocrine cells and peptidenergic nerve fibers were seen in the glands and muscle tissue (Fig. 3d). SER-positive endocrine cells were also detected.

GHR-positive endocrine cells and inflammatory cells were found in glands and around them in all specimens (Fig. 3e). We could find also ghrelin immunoreactivity in the intramural nerve ganglionic cells.

DISCUSSION

The gallbladder- biliary pathways system played an important role in modulating bile flow, secreting mucous substances and maintaining biliary motility [35]. Previous studies reportedvasointestinal polypeptide (VIP), SP, somatostatin and met-enkephalinIR nerves in the gall bladder and biliary pathway of the guinea pig [12]. Their secreted hormones exerted neuronal control on biliary motility [36].

In the present study it was showed for the first time that ghrelin-positive endocrine cells existed in swine gallbladder, ductus cysticus and choledochus. The presence of SOM-, SER-, GAS- and CHA-positive endocrine cells was also described there.

Endocrine cells were most numerous in cystic duct followed by common bile duct and were less in the neck region of the gallbladder. Our unpublished observations showed single endocrine cells in human gallbladder, and plenty of endocrine cells in the lower third of human common bile duct in mechanic jaundice [7].

We have found SOM-positive endocrine cells in all studied sites located in the mural glands and in the surface epithelium.

SOM IR endocrine cells were found in human extrahepatic bile ducts [5, 7]. SOM-positive D cells were detected in common bile duct of guinea pig [12] and bile duct of pigs [36]**.** Unlike *Sand et al., 1993* we detected SOM-positive nerve fibers in bile ducts. It could be suggested that SOM is a biliary neurotransmitter.

SOM played mainly an inhibitory role in endocrine secretion, biliary motility etc. [12]. In the guinea pig there had been demonstrated lack of effect of SOM on the motility of the gallbladder [37].

SOM that was a neuropeptide and hormonehad been reported to inhibit spontaneous and CCK-induced gallbladder motility [38]. The authors showed that when the gallbladder smooth muscle was contracted by electrical stimulation, SOM decreasedthe contraction by suppressing acetylcholine release.

SOM was known to inhibit the effect of some peptide hormones such as CCK that induced relaxation of the sphincter of Oddi [39]. In diabetes, SOM IR ECs increased in biliary pathways and the increased hormone release induced contraction on sphincter of Oddi muscles and concomitant bilestasis and stone formation [19]**.** SOM stimulated sphincter of Oddi activity that favoured the reduced flow of bile into the duodenumand facilitated gallbladderfilling [40]**.**

Single SER-positive endocrine cells were observedin the intramural glands in gallbladder neck and in the ductus cysticus and choledochus. SER IR endocrine cells were found to be exclusively low in number in the rat pancreatic and bile duct system [19] in pig bile duct and gallbladder [36] or missing in rat bile duct [18]. In a previous study we described that SER- positive endocrine cells increased in number in chronic cholangitis [7]. Serotinin is an important neurohumoral signaling molecule [13]**.** SER-positive endocrine cells are sensory transducers that responded to mechanical or chemical stimulation of the mucosa by releasing serotonin [41] that regulated visceral sensation and gut motility [13]**.**

Gastrin-positive endocrine cells were found in cystic duct and choledochal duct in our study. Something more, GAS-positive nerves were detected in these structures. In a previous study [7] it was observed G cells in human common bile duct, whose ultrastructure was similar to that of gastric G cells. Our present finding confirmed our previous suggestion that GAS-positive (G cells) were part of the gastrointestinal triangle. The latter consisted in GAS-positive cells in the junction of the cystic duct with common bile duct, the junction between the second and the third portions of the duodenum and by the junction between the neck and body of the pancreas [42]. In humans and pigs G cells were most numerous in antrum and stimulated gastric acid secretion [43, 44]. Presence

of G cells and GAS-positive nerve fibers in biliary structures could be explained with initiating contraction of their smooth muscle cells and with modulation of their motility [45]**.**

Ghrelin was mainly described in endocrine cells of human digestive, respiratory and urinary system and in particular cells of some endocrine glands [27]. In human stomach (oxyntic region) the relative percentages of main four endocrine cell types were 30% for ECL cells (histamine-positive), 20% for P/D_1-like cells (GHR), 22% for D cells (SOM), and 7% for EC cells (SER) [46]. GHR-positive cells accounted for 23% of CHA-positive cells there [46]. It was established that GHR-positive cells co-localized with CHA and VMAT2 [27, 46]. In pig's stomach GHR-positive cells encountered about 84. 79 cells/mm^2 in corpus (the greatest number) and showed decreased frequency in cardia, pylorus and small intestine [47].

Endocrine cells in biliary system were rarely described in animals [18, 19, 36] and in humans [7]. There were no data about GHR-positive cells in human biliary pathways [20] and in pig's biliary structures. Our study demonstrated GHR-positive endocrine cells in glands and GHR positivity in some nerve fibers and ganglionic cells in gallbladder, cystic duct and choledochal duct in pigs. In human's gallbladder there was detected ghrelin and ghrelin receptor mRNA expression [34] but ghrelin tissue localization wasn't described yet. Based on our finding of GHR presence in nerve structures of biliary pathways, ghrelinneurotransmitter function could be supposed. There existed data about ghrelin localization in central nervous system [48] and in Auerbah nerve plexus [26, 49]. GHR had structural similarities with motilin [50] and co-existed in one and the same cell. It was reported that motilin initiated and realized gallbladder emptying [50] and stimulated contraction of smooth muscle cells in human gallbladder [51]. Ghrelin on its side was a growth hormone-secretagogue and the last increased gallbladder motility [52]. It was shown that in humans low ghrelin serum levels were seen in metabolic syndrome and in gallstone disease [53]. Ghrelin receptors might exist on mucosal cells and signals for GHR production might come from the lumen. Another explanation for GHR presence in biliary mucosal endocrine cells might be for its role in stimulating the growth hormone secretion that is necessary for cell proliferation.

We had demonstrated ghrelin positivity in some immune cells in biliary pathways.

It was already shown that GHS-RmRNAexpression was found in human lymphoid organs [54] or in purified human T cells [55]. The majority of T cells from human donors express and secrete low levels of ghrelin constitutevely and high levels upon cellular activation [55]. Therefore our finding of Ghr positivity in some immune cells in pig's biliary pathways confirmed the presence of basal ghrelin expression in some inflammatory cells. The endogenous GR inhibited proinflammatory cytokine expression (IL-1a, IL-1b, IL-6) [55] and it would be supposed tht Ghr may function as an important signal modulator among the endocrine, nervous and immune systems. The presence of GHR inendocrine andimmune cells in biliary pathways confirmed the integrity between this hormone and immune reactions in organism.

REFERENCES

1. F. Sandler, R. Hakanson, *Elsevier*, **6**, 219 (1988).
2. A. Andrew, B. Kramer, B.B. Rawdon, *J Pathol*, **186**, 117 (1998).
3. T. Roskams, V. Desmet, *The Anatomical record*; **291**, 628 (2008).
4. H. Kurumaya, G.F. Ohta, K. Nakanuma, *Arch Pathol Lab Med*, **113**, 143 (1989).
5. H. Dancigier, U. Klein, U. Leuschner , K. Hubner, M. Classen, *Gastroenterology*, **86**, 892 (1984).
6. M.P. Hoang, L.A. Muracata, A.L. Pedilla-Rodriguez, J. Albores-Saavedra, *Mod Pathol*, **14**, 1119 (2001).
7. M.V. Gulubova, P. Hadjipetkov, D. Sivrev, G. Ilieva. *Hepato-Gastroenterology*, **59** (2012).
8. J. Ducla-Soares, M. Ferreira, C. Campos, *Am J Gastroenterol*, **87**, 668 (1992).
9. J.C.Doss, C.C.Grone, T.J. Rosol. *Vet Pathol*, **35**, 312 (1998).
10. F.J.Esteban, J.B.Jimenz, J.A.Barroso, M.L.Pedrosa, J. Del Moral, J.Rodrigo, MA.Peinado. *J Anat*, **193**, 241(1998).
11. P. Chen, B. Hu, Q. Tan, L. Liu, D. Li, C. Jiang, H. Wu, J. Li, C. Tang. *NeurogastroenterolMotil*, **22**, 935 (2010).
12. W-Q.Cai, G.Gabella. *J Anat*, **136**, 97 (1983).
13. H.Zhao, I.Sovadinova, V.M.Swope, G.M.Swain, M.M.Kadrofske, X.Bian. *NeurogastroenterolMotil*, **23**, 161 (2011).
14. A.K.Patnaik, P.H.Liberman, R.A.Erlandson, C.Antoanescu. *VetPathol*, **42**, 331 (2005).
15. M.Y. Lee. *J Cathol.Med.Coll*, **41**, 787 (1988).
16. P. Heitz, J.M. Polak, C.M. Kasper, A.G.E. Pearse, *Histochemistry*, **50**, 319 (1977).
17. R.T. Gemmell, T. Heath, *J Anat*, **115**, 221 (1973).
18. I-S.Park, M. Bendayan, *The Anatomical Record*, **232**, 247 (1992).
19. I-S. Park, M. Bendayan, *Pancreas*, **9**, 566 (1994).
20. J.R. Dutton, N.L. Chillingworth, D. Eberhard, C.R. Brannon, M.A. Hornsley, D. Tosh, J.M.W Slack, *J Cell Sci*, **120**, 239 (2006).

21. M. Grönberg, A.V. Tsolakis, L. Magnusson, E.T. Janson, J. Saras, *J HictochemCytochem*, **56**, 793 (2008).
22. T. Hayashida, K. Nakahara, M.S. Mondal, Y. Date, M. Nakazato, M. Kojima, K. Kangawa, N. Murakami, *Journal of Endocrinology*, **173**, 239(2002).
23. C-M. Zhao, M.A. Furnes, B. Stenstrom, B. Kulseng, D. Chen, *Cell Tissue Res*, **331**, 575 (2008).
24. M. Kojima, H. Hosoda, Y. Date, M. Nakazato, H. Matusio, K. Kangawa, *Nature*, **402**, 656 (1999).
25. M. Kojima, A. Kanagawa, *Nat ClinPractEndocrinolMetab*, **2**, 80 (2006).
26. Sakata I, Sakai T. Ghrelin, *Int J Pept*, **7** (2010).
27. G. Rindi, V. Necchi, A. Savio, A. Torsello, M. Zoli, V. Locatelli, F. Raimondo, D. Cocchi, E. Solcia, *Histochem Cell Biol*, **117**, 511 (2002).
28. C. Tomasetto, S.M. Karam, S. Ribieras, R. Masson, O. Lefebvre, A. Staub, G. Alexander, M-P. Chenard, M-C.Rio, *Gastroenterology*, **119**, 395 (2000).
29. Y. Date, M. Nakazato, S. Hashiguchi, K. Dezaki, M.S. Mondal, H. Hosoda, M. Kojima, K. Kangawa, T. Arima, H. Matsuo, T. Yada, S. Matsukura, *Diabetes*, **51**, 124 (2002).
30. M. Volante, E. Allia, E. Fulcheri, P. Cassoni, E. Ghigo, G. Muccioli, M. Papotti, *Am J Pathol*, **162**, 645 (2003).
31. G. Muccioli, M. Papotti, V. Locatelli, E. Ghigo, R. Deghenghi, *J Endocrinol Invest*, **24**, RC7 (2001).
32. G. Rindi, A. Torsello, V. Locatelli, E. Solcia. *ExpBiol Med*, **229**, 1007 (2004).
33. N. Hattori, T. Saito, T. Yagyu, B.H. Jiang, K. Kitagawa, C. Inagaki, *J ClinEndocrinolMetab*, **86**, 4284 (2001).
34. S. Gnanapavan, B. Kola, S.A. Bustin, D.G. Morris, P. McGee, P. Fairclough, S. Bhattacharya, R. Carpenter, A.B. Grossman, M. Korbonits, *J ClinEndocrinolMetab.Jun*, **87**, 2988 (2002).
35. J.P. Ryan. Motility of the gallbladder and biliary tree. In: Physiology of the Gastrointestinal Tract. Jonson, LR, ed. Raven Press, New York, 1987, 665.
36. J. Sand, H. Tainio, I. Nordback, *Dig Dis S*ci, 38, 694 (1993).
37. P. Poitras,T. Yamada, J.H. Walsh, *Journal of Physiology and Pharmacology*, **58**, 179 (1979).
38. K. Milenov, R. Rakovska, R. Kalfin, P. Mantovani. *Nuropeptides*, **17**, 75 (1990).
39. G.P. Adami, R. Leandri, J.C. Sarles, *GastroenterolClinBi*ol, **10**, 108 (1986).
40. J. Huang, R.T.A. Padbury, A.C. Schloithe , M.R. Cox, M.E. Simula, J.R. Harvey, R.A. Baker, J. Toouli, G.T.P. Saccone, *Gastroenterology*, **115**, 672 (1998).
41. .K. Racke, A. Reiman, H. Schoworer, H. Kilbinger. *Behav Brain Res*, **73**, 83 (1996)
42. B.E. Stabile, D.J. Morrow, E. Passaro, *Am J Surg*, **147**, 25 (1984).
43. E. El-Omar, I. Penman, C.A. Dorrian, J.E.S. Ardill, K.E.L. McColl, *Gut*, **34**, 1060 (1993).
44. J.C.P. Silva, J.L. Santos, A.J.A. Barbosa, *J Comp Path*, **126**, 235 (2002).
45. M.R. Cox, R.T.A. Padbury, T.L. Snelling, A.C. Schloithe, J.R. Harvey, J. Toouli, G.T.P. Saccone. *Dig Dis Sci*, **43**, 1275 (1998).
46. Y. Date, M. Kojima, H. Hosoda, A. Sawaguchi, M.S. Mondal, T. Suganuma, S. Matsukura, K. Kangawa, M. Nakazato, *Endocrinology*, **141**, 4255 (2000).
47. F. Vitari, A. Di Giancamillo, D. Deponti, V. Carollo, C. Domeneghini. *Vet Res Commun*, **36**, 71 (2012).
48. S. Lu, J.L. Guan, Q.P. Wang, K. Uehara, S. Yamada, N. Goto, Y. Date, M. Nakazato, M. Kojima, K. Kangawa, S. Shioda. *Neurosci Lett*, **321**, 157 (2002).
49. L. Xu, I. Depoortere, C. Tomassetto, M. Zandecki, M. Tang, J.P. Timmermans, *RegulPept*, **124**, 119 (2005).
50. B. De Smet, A. Mitselos, I. Depoortere, *PharmacolTher*, **123**, 207 (2009).
51. T.Yamasaki, K.Chijiiwa, Y.Chijiiwa. *J Surg Res*, **56**, 89 (1994).
52. A. Moschetta, T.B. Twickler, J.F. Rehfeld, N. A.van Ooteghem, M.C. Cabezas, P. Portincasa, G. P.van Berge-Henegouwen, K.J. van Erpecum, *Dig Dis Sci*, **49**, 529 (2004).
53. N. Mendez-Sanchez, G. Ponciano-Rodriguez, L. Bermejo-Martinez, A.R. Villa, N.C. Chavez-Tapia, D. Zamora-Valdes, R. Pichardo-Bahena, B. Barredo-Prieto, M.H. Uribe-Ramos, M.H. Ramos, H.A. Baptista-Gonzalez, M.Uribe. *World J Gatroenterol*, **12**, 3096 (2006).
54. S.Gananapavan, B.Kola, S.A.Bustin, D.G.Morris, P. McGee , P. Fairclough, S. Bhattacharya, R. Carpenter, A.B. Grossman, M. Korbonits. *J ClinEndocrinolMetab*, **87**, 2988 (2002).
55. V.D.Dixit, E.M.Schaffer, R.S.Pyle, G.D.Collins, S.K.Sakthivel, R.Palaniappan, J.W. Jr Lillard, D.D.Taub, *J Clin Invest*.,**114**, 57 (2004).

ЕНДОКРИННИ КЛЕТКИ В ЖЛЪЧНИЯ МЕХУР, DUCTUS CYSTICUS И DUCTUS CHOLEDOCHUS ПРИ ПРАСЕТА СЪС СПЕЦИАЛНО ОТНОШЕНИЕ КЪМ ГРЕЛИНА

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(Резюме)

Съществуват няколко съобщения, в които са описани ендокринните клетки (ECs) в жлъчният мехур и жлъчните пътища при човека, свързани с хронично възпаление. При прасето обаче ние не открихме данни за присъствието ECs в тези структури. Грелинът е пептиден хормон, участващ в освобождаването на растежния хормон и в модулирането на приема на храна. Той притежава също проинфламаторни функции. Грелин позитивните ECs са основен източник на грелин. Настоящото изследване разкрива имунохистохимично присъствието на грелин+ ECs в жлъчния мехур и *ductus choledochus.* Ендокринните клетки в жлъчния мехур са твърде малко. Наблюдавани са единични Chromogranin A^+, Somatostatin$^+$ и Serotinin$^+$ECs. В *ductus choledochus са* установени по-голям брой Chromogranin A^+, Somatostatin$^+$ и Serotinin$^+$ECs в сравнение с жлъчния мехур. Най-много ECs бяха локализирани в *d.cysticus*. Те са също Chromogranin A^+, Somatostatin$^+$, Gastrin$^+$ и Ghrelin$^+$ECs. В заключение, предполагаме, че ECs, включително грелин позитивните ECs упражняват действие върху физиологичните и патологичните състояния в жлъчните пътища при прасета.