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Original article

Distribution and chemical coding patterns of cocaine- and amphetamine-regulated transcript-like immunoreactive (CART-LI) neurons in the enteric nervous system of the porcine stomach cardia

W. Rękawek[1], P. Sobiech[1], S. Gonkowski[2], K. Żarczyńska[1], A. Snarska[1], T. Waśniewski[4], J. Wojtkiewicz[3]

[1] Department and Clinic of Internal Diseases, Faculty of Veterinary Medicine, Oczapowskiego 14, 10-719 Olsztyn, Poland
[2]Department of Clinical Physiology, Faculty of Veterinary Medicine, University of Warmia and Mazury, Oczapowskiego 13, 10-719 Olsztyn, Poland
[3] Department of Neurology and Neurosurgery, Stem Cell Research Laboratory, Faculty of Medical Sciences, University of Warmia and Mazury, Warszawska 30, 10-082 Olsztyn, Poland
[4] Department of Gynecology and Obstetrics, Faculty of Medical Sciences, University of Warmia and Mazury, Żołnierska 18, 10-561 Olsztyn, Poland

Abstract

The aim of this study was to determine the presence of cocaine- and amphetamine-regulated transcript-like immunoreactive (CART-LI) neurons and co-localisation of CART with vesicular acetylcholine transporter (VAChT), neuronal nitric oxide synthase (n-NOS), vasoactive intestinal polypeptide (VIP), substance P (SP) and leu-enkephalin (LENK) in the enteric nervous system of the porcine gastric cardia by using a double-labelling immunofluorescence technique. CART-LI neurons were observed in the myenteric plexus (18.2±2.6%). A dense network of CART-LI nerve fibers was mainly observed in the muscular layer. CART showed co-localization mainly with VAChT, n-NOS, VIP and to a lesser degree with LENK and SP. Distribution of CART and its co-localization with other neurotransmitters suggest that this peptide plays an important role in gastric motility in the pig.

Key words: Cocaine- and amphetamine-regulated transcript (CART), enteric nervous system (ENS), stomach, immunohistochemistry, pig

Correspondence to: P. Sobiech, e-mail: psobiech@uwm.edu.pl

Introduction

Cocaine- and amphetamine-regulated transcript (CART), a polypeptide consisting of 116 or 129 amino acid residues, was described for the first time in 1981, when it was isolated from the sheep hypothalamus (Spiess et al. 1981, Douglass et al. 1995).

The expression of CART mRNA in the rat striatum increases dramatically after administration of high doses of cocaine and amphetamine (Douglass et al. 1995) and for this reason, the discussed peptide was termed the cocaine- and amphetamine-regulated transcript (CART). Previous studies revealed the presence of CART both in the central and peripheral nervous system, including primary sensory neurons (Dun et al. 2000), cholinergic nerves of pancreatic islets (Jensen et al. 1999), intramural ganglia of the urinary bladder trigone (Zacharko-Siembida and Arciszewski 2014a) and neurons supplying the adrenal and thyroid glands (Wierup et al. 2007).

CART can play diverse roles in the organism, such as participation in the stress response, conducting sensory stimuli and feeding behavior (Douglass et al. 1995, Kuhar and Dall Vechia 1999), as well as in ontogenesis of the nervous system (Risold et al. 2006).

CART was also described within the enteric nervous system (ENS), which, apart from extrinsic sympathetic and parasympathetic innervation of the gastrointestinal (GI) tract (Skobowiat et al. 2010, Luyer et al. 2013), plays a crucial role in the control of all intestinal functions. The ENS comprises of millions of neurons, and its construction depends on the segment of GI tract and animal species. In the intestines of large mammals (such as the pig), neurons of the ENS are grouped into three intramural ganglionated plexuses: the myenteric plexus (MP) - located between the longitudinal and circular muscle layers, the outer submucous plexus (OSP) - found near the circular muscle layer, and the inner submucous plexus (ISP) - distributed between the muscularis mucosa and lamina propria (Kaleczyc et al. 2007, Gonkowski et al. 2010). In contrast, gastric enteric neuronal cells form visible myenteric plexus, whereas within the submucosa only single ganglia (SG), which do not form typical enteric plexuses, were described (Zacharko-Siembida and Arciszewski 2014b).

Previous studies demonstrated the presence of CART-LI structures in different segments of the gastrointestinal tract of various mammal species, including humans (Ekblad 2006, Gunnarsdottir et al. 2007, Wierup et al. 2007, Arciszewski et al. 2009, Gonkowski et al. 2009a,b, 2012, Kasacka et al. 2012, Janiuk et al. 2013, Zacharko-Siembida and Arciszewski 2014b). However, till now, knowledge of the exact functions of CART in the GI tract is rather scanty. Previous studies suggest that CART can inhibit the nitric oxygen-induced relaxation of intestinal muscles (Ekblad et al. 2003).

The aim of the present study was to determine the distribution of CART-LI nerve structures, as well as co-localization of CART with other neurotransmitters within the gastric ENS in the pig, which is becoming a very important laboratory animal considering its many well-known similarities to humans in anatomical, histological, biochemical and physiological properties (Brown and Timmermans 2004, Verma et al. 2011).

Materials and Methods

The experiment was performed on five female Polish Large White piglets aged approximately eight weeks, with body weight of 12 to 18 kg. The animals were obtained from a commercial farm in north-eastern Poland. Directly before tissue sampling, the animals were kept under standard laboratory conditions, with special attention paid to minimizing the stress reaction. All experimental procedures were approved by the Local Ethics Commission of the University of Warmia and Mazury in Olsztyn (no. 28/2008).

The animals were euthanized through intravenous administration of sodium thiopental at a dose of 20 mg/kg of body weight (Thiopental, Sandoz, Kundl-Rakusko, Austria). Transcardial perfusion was performed using 4% buffered paraformaldehyde (pH 7.4).

Samples, of approximately 1 cm^2 (one from each animal), were collected from the *pars cardiaca ventriculi,* near the cardiac orifice, within the (so-called) *pars nonglandularis,* close to the edge of the diverticulum. The tissue pieces were kept for 15 minutes in the same fixing solution and after washing in phosphate buffered saline for three consecutive days, were transferred into 18% sucrose solution in which they were kept until handling. Prior to cutting, the material was mounted on a metal table by using OCT compound and allowed to freeze at -25°C. Ten-fm-thick cryostat sections were processed for double-labeling immunofluorescence as described previously by Gonkowski et al. (2010). The sections were incubated overnight in the humid chamber, with different species primary antibodies against PGP 9.5 (used here as panneuronal marker), CART, vesicular acetylcholine transporter (VAChT), substance P (SP), neuronal-nitric oxide synthase (n-NOS), vasoactive intestinal polypeptide (VIP) and leu-enkephalin (LENK) (for details, see Table 1). The resulting immunological complexes were visualized with species-specific secondary antibodies labeled with FITC or biotin and,

Table 1. Specification of immune reagents.

Primary antibody				
Antisera	Code	Host Species	Dilution	Supplier
PGP9.5	7863-2004	Mouse	1:2000	Biogenesis Inc, UK www.biogenesis.co.uk
CART	H-003-61	Rabbit	1:22000	Phoenix Europe www.phoenixpeptide.com
NOS	N2280	Mouse	1: 1000	Sigma, US; www.sigma-aldrich.com
VIP	9535-0504	Mouse	1: 3600	Biogenesis Inc, UK www.biogenesis.co.uk
SP	8450-0505	Rat	1:300	Biogenesis Inc, UK www.biogenesis.co.uk
LENK	4140-0355	Mouse	1: 500	Biogenesis Inc, UK www.biogenesis.co.uk
VAChT	H-V007	Goat	1: 6000	Phoenix Europe www.phoenixpeptide.com
Secondary antibodies				
Reagent			Dilution	Supplier
FITC-conjugated donkey-anti-mouse IgG (H+L)			1:800	Jackson, 715-095-151
FITC-conjugated donkey-anti-rat IgG (H+L)			1:800	Jackson, 712-095-153
FITC-conjugated donkey-anti-goat IgG (H+L)			1:1000	Jackson, 705-096-147
Biotinylated goat anti-rabbit immunoglobulins			1:1000	DAKO, E 0432
CY3- conjugated Streptavidin			1:9000	Jackson, 016-160-084

after another rinse cycle, were visualized with streptavidin-CY3. Double labeled perikarya were visualized under an Olympus BX51 microscope equipped with epifluorescence filter sets, and counted.

To ensure reliability of the results, pre-absorption control of antibodies was performed.

In control staining, specific antiserum was first incubated with appropriate antigen (10-100 µg of blocking antigen per 1ml of diluted antiserum). No specific immunostaining was observed in control probes.

In the first phase of the experiment, the percentage of CART-LI neurons was counted by estimation of co-localization of CART-LI and PGP 9.5-LI neurons. At least 1000 PGP 9.5-LI neurons were counted. To determine co-localization of CART with other substances studied, the percentage of n-NOS-, VIP-, SP-, VAChT- and LENK-LI cells in relation to all populations of CART-positive neurons was counted in each of the enteric ganglia. Only neurons with a visible nucleus were taken into account. The results were presented as means ± SEM. All calculations were performed using GraphPad Prism 3 software (GraphPad Software, La Jolla, CA, USA). To prevent double counting of neurons, only sections separated by a distance of 200 µm were used for the analysis.

For semi-quantitative evaluation of the density of intraganglionic CART-LI nerve fibers, an arbitrary scale was used, where (-) means the absence of fibers and (++++) depicts a very dense meshwork of fibers. A semi-quantitative evaluation of the density of nerve fibers within the mucosal layers was based on a count of all profiles immunoreactive to a given antigen per observation field. Nerve profiles were counted in 4 sections per animal (in 5 fields per section) and the data obtained were pooled and presented as a mean. To ensure the reliability of the results, statistical analysis of the results obtained from individual animals was performed (one-way analysis of variance followed by Bonferoni's *post-hoc* test, a $p \leq 0.05$ was considered significant).

Results

CART-LI neurons were present in the muscular layer (Fig. 1a, 1a') of the porcine gastric wall. A small number of CART-LI neurons was observed below the muscle layer (Fig. 1b, 1b'); they are transferred neurons of muscle layer, and therefore were qualified to the population of MP. In the muscular layer, neurons created well visible myenteric plexuses, which usually consisted of 6 to 14 neurons each. The largest number of CART-LI perikarya were visualized in MP (Fig. 1a'), where these cells amounted to 18.2 ± 2.6% of all cells immunostained to PGP 9.5. Moreover, the present study revealed the occurrence of a dense network of CART-LI nerve fibers (+++) in the MP

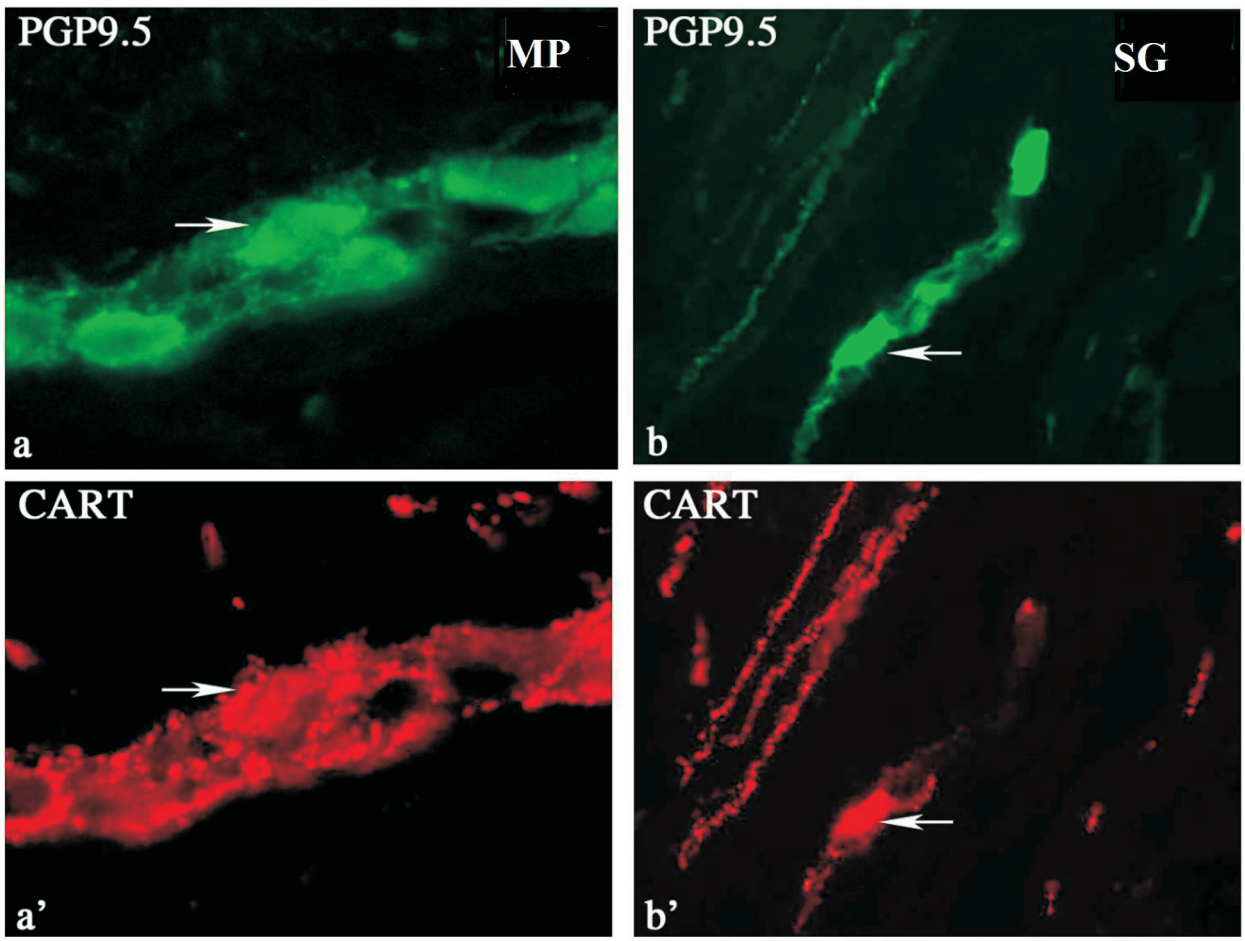

Fig. 1. Enteric plexuses of the porcine stomach cardia immunostained for PGP 9.5 and CART; a, a' - myenteric plexus (MP), b, b'- myenteric neurons transferred to submucosa. Co-localization of both antigens in the perikarya indicated with arrows. Bar = 25 μm

Table 2. Co-localization of CART with n-NOS, VIP, LENK, VACHT, SP in neurons.

Co-localization	MP (*)
CART/n-NOS	67.7 ± 2.4
CART/VIP	48.6 ± 3.1
CART/LENK	16.9 ± 2.7
CART/VACHT	91.6 ± 4.8
CART/SP	14.7 ± 2.1

MP - myenteric plexus, CART-LI neurons expressing studied neurotransmitter in % (mean ± SEM) of all CART-positive cells.

(Fig. 1a') and a very high density of CART-LI fibers in the longitudinal and circular muscles (++++) (Fig. 3). In the submucosal layer, the density of nerve processes was significantly lower (+).

The present study revealed the co-localization of CART with n-NOS, VIP, LENK, VAChT and SP in enteric neurons of the gastric cardia (Table 2, Figs. 2, 3). In the MP, the number of CART-LI neurons simultaneously immunopositive to VAChT amounted to 91.6%. For other neuronal factors studied, these values were lower and amounted to over 67.7% for n-NOS, about 48.6% for VIP, almost 17% for LENK and above 14% for SP (Table 2). Statistical analysis revealed no significant differences in the results obtained from individual animals.

Discussion

The present study revealed CART-LI nervous structures in the wall of the porcine stomach. These

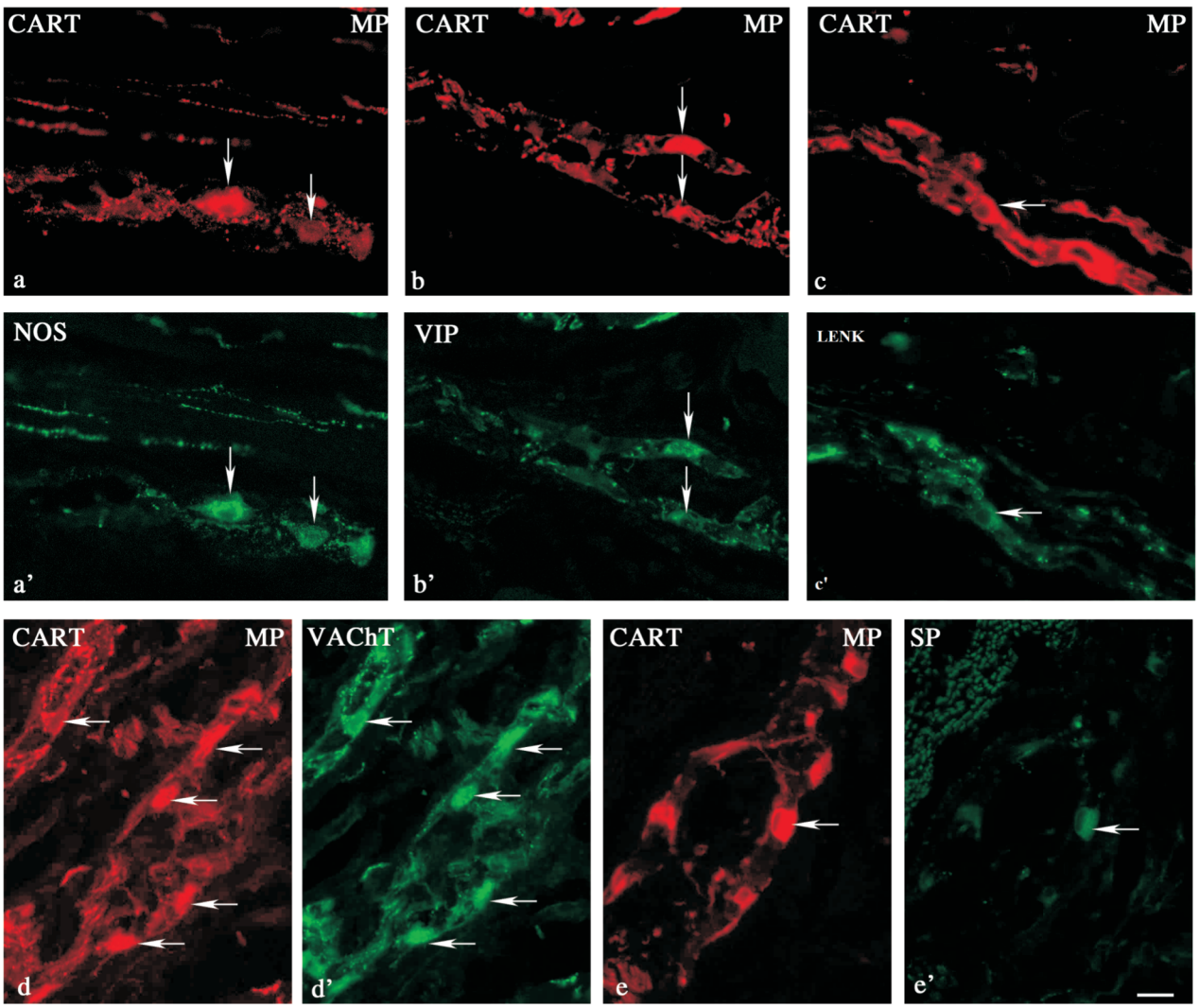

Fig. 2. Myenteric plexus of the porcine stomach cardia, immunostained for CART and NOS, VIP, LENK, VAChT or SP. Co-localisation of the antigens in the perikarya indicated with arrows a, a'- co-localisation of CART and n-NOS, b,b'- co-localisation of CART and VIP, c, c' - co-localisation of CART and LENK, d, d' - co-localisation of CART and VAChT, e, e' - co-localisation of CART and SP. Bar = 25 µm

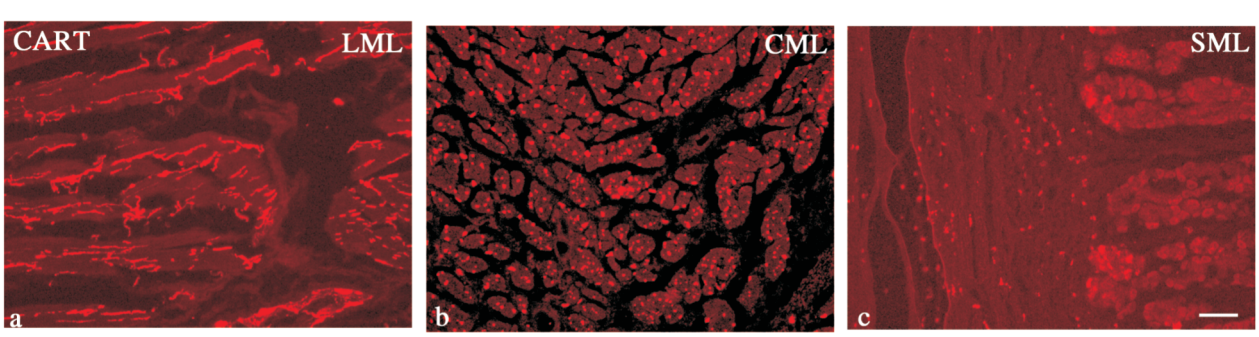

Fig. 3. Distribution of nerve fibers immunostained for ART within the longitudinal (LML; a), circular muscle (CML; b) and submucosal layer (SML; c) of the porcine stomach cardia. Bar = 50 µm

data are in agreement with previous reports showing the presence of CART in the GI tract (Couceyro et al. 1998, Ekblad et al. 2003, Ellis and Mawe 2003, Wierup et al. 2007, Gunnarsdóttir et al. 2007, Gonkowski et al. 2009a,b, Arciszewski et al. 2009, Janiuk et al. 2013, Zacharko-Siembida and Arciszewski 2014b). The most numerous CART-LI nervous structures were observed in the MP, which is in agree-

ment with previous findings (Zacharko-Siembida and Arciszewski 2014b) and can suggest that this peptide plays a role in the regulation of gastrointestinal motility. Unfortunately, till now the direct influence of CART on the intestinal muscle *in vitro* has not been verified (Ekblad et al. 2003). The percentage of CART-positive neurons observed in the porcine stomach cardia is higher than the number of such cells within the large intestine of this species, where CART-LI myenteric neurons amounted to only from 2% to 8% (Gonkowski et al. 2009b), but this is similar to the values related to the small intestine (Wojtkiewicz et al. 2012). Moreover, in the present study CART-LI neurons were observed in the submucosal ganglia. Previous investigations described such cells in porcine intestines (Gonkowski et al. 2009b, Wojtkiewicz et al. 2012), but not in the stomach (Zacharko-Siembida and Arciszewski 2014b).

Differences in the number of CART-LI nervous structures in the submucous and mucosal layer between those found in the present study and those reported in previous investigations could be explained by the fact that this experiment was performed on young animals, whereas some previous studies (Wierup et al. 2007) were conducted on adult pigs. These observations suggest that the role played by CART in the gastrointestinal system could change with individual development of the organism and such a thesis is supported by reports claiming that the number of CART-LI neurons changes during ontogenesis (Risold et al. 2006). Other studies (Zacharko-Siembida and Arciszewski 2014b), where CART-LI neurons in SG were not found, were performed on another race of pigs (Landrace x Pietrain) which suggests that there are differences in the distribution of CART-positive structures in the gastrointestinal tract of different breeds of pigs. Moreover, some differences in CART-immunoreactivity of gastric nervous structures can result from different feeding.

On the other hand, previous observations of CART-LI nerve structures in the porcine intestines (Gonkowski et al. 2009a,b, Wojtkiewicz et al. 2012) and the present results are in line with data obtained in humans (Gunnarsdóttir et al. 2007, Kasacka et al. 2012), which testifies to the well-known similarities between human and porcine organisms (Brown and Timmermans 2004, Verma et al. 2011).

So far, very little is known about the exact functions of CART within the porcine ENS. In previous investigations this peptide was described as an inhibitor of gastric acid secretion (Okumura et al. 2000), inhibitor of NO-mediated colonic relaxation (Ekblad et al. 2003), as well as a reducer of colonic motility via cholinergic pathways (Tebbe et al. 2004). Moreover, some investigations have revealed changes in the number of CART-LI structures in the ENS during various pathological processes (Gunarsdóttir et al. 2007, Gonkowski et al. 2009a, 2012) which suggests neuroprotective functions of this peptide. However, CART still remains an obscure substance and further studies are needed to reveal in detail its roles within the gastrointestinal tract.

The present investigation revealed the co-localization of CART with VAChT, SP, LENK, VIP and n-NOS in neurons of the ENS in the porcine stomach cardia.

The majority of CART-LI myenteric neurons (92%) were also VAChT-positive, which indicates their cholinergic function. This is in agreement with previous studies on guinea pig intestines, where co-existence of these substances amounted to 82% (Ellis and Mawe 2003). Acetylcholine is a well-known neuromediator in the ENS. It fulfils various functions in the gastrointestinal tract, ie. it mediates motility and secretory reflexes (Harrington et al. 2010) or regulates the innate immune response (Bonaz et al. 2013). The excitatory effects on intestinal motility seem to be the most important (Olsson and Holmgren 2001).

A large number of CART-LI neurons were also n-NOS-positive (about 68%) Surprisingly, nitrergic neurons in the ENS play a contrasting role to cholinergic innervation. The nitric oxide is mainly present in inhibitory motor neurons and causes relaxatory effects in the intestinal muscles (Ellis and Mawe 2003, Bagyánszki et al. 2010). n-NOS is also a well known mediator of hormone secretion (Sayegh and Ritter 2003), peristaltic reflexes (Grider 1992), gut mucosal protection (Di and Krantis 2002) and neuroprotective factor (Lin et al. 2004). Co-localization of CART with neurotransmitters, which are markers of nerve structures with opposing functions, may suggest that CART acts as a neuromodulator of neurotransmission in both excitatory and inhibitory neurons. A similar situation with regard to other neurotransmitters and/or neuromodulators has been observed in the guinea pig, in which, in both cholinergic and nitrergic enteric neurons, the same various neuronal factors, such as neuropeptide Y (NPY), VIP, SP and /or LENK were determined (Schemann et al. 1995).

Nearly half of the CART-LI myenteric neurons observed during the present investigation were also VIP-positive. This is in agreement with previous studies on the porcine (Wierup et al. 2007, Wojtkiewicz et al. 2012) and rodent gastrointestinal tract (Ekblad et al. 2003, Ellis and Mawe 2003). VIP is a mediator responsible for smooth muscle relaxation, stimulation of intestinal fluid and elecrolyte secretion (Grider et al. 1992), and regulation of blood flow in the digestive

tract (Anderson 1984). VIP is also an important neuroprotective and anti-inflammatory agent in the ENS (Abad et al. 2003, Sandgren et al. 2003). Therefore, the co-localization of CART and VIP in enteric neurons observed in previous studies (Ekblad 2006, Wierup et al. 2007, Wojtkiewicz et al. 2012) and in the present investigation, as well as an increase in CART-like immunoreactivity in various pathological processes in the gastrointestinal system (Gonkowski et al. 2009a, 2012) suggest that CART participates in neuroprotective processes within the ENS.

The present study revealed co-localization of CART and LENK or SP in neurons. SP is a neuropeptide known as one of the most important mediators responsible for conduction of sensory stimuli, and the development and course of inflammatory bowel disease (IBD) (Koon and Pothoulakis 2006). It causes rapid contraction of smooth muscle in the guinea pig jejunum and rat duodenum (Chang and Leeman 1970). The main source of SP in the GI tract are muscularis and submucosal neurons, intrinsic sensory neurons and neurons in dorsal root ganglia (Holzer 1997).

Leu-enkephalin, one of the endogenous opioids, also has an effect on gastrointestinal motility by inhibiting the release of acetylcholine, which consequently reduces the motility of the GI tract (Porcher et al. 2000). Co-localization of CART with LENK suggests that the roles of these neuronal factors within the ENS are at least partly synergistic.

To sum up, the results obtained suggest that the functions of CART are not limited only to the control of gastrointestinal tract motility but also can include other roles such as control of gastrointestinal secretion, immunological reactions and development of inflammatory processes. On the other hand, many aspects of co-localization of CART with other neuronal factors in the ENS, as well as functions of CART within the gastrointestinal tract, still remain obscure and further physiological and genetic investigations are needed.

References

Abad C, Martines C, Juarranz MG, Arranz A, Leceta J, Delgado M, Gomariz RP (**2003**) Therapeutic effects of vasoactive intestinal peptide in the trinitrobenzene sulfonic acid mice model of Crohn's disease. Gastroenterology 124: 961-971.

Andersson PO (**1984**) Vascular control in the colon and rectum. Scand J Gastroenterol Suppl 93: 65-78.

Arciszewski MB, Barabasz S, Skobowiat C, Maksymowicz W, Majewski M (**2009**) Immunodetection of cocaine- and amphetamine- regulated transcript in the rumen, reticulum, omasum and abomasum of the sheep. Anat Histol Embryol 38: 62-67.

Bagyánszki M, Krecsmarik M, De Winter BY, De Man JG, Fekete E, Pelckmans PA, Adriaensen D, Kroese AB, Van Nassauw L, Timmermans JP (**2010**) Chronic alcohol consumption affects gastrointestinal motility and reduces the proportion of neuronal NOS-immunoreactive myenteric neurons in the murine jejunum. Anat Rec (Hoboken) 293: 1536-1542.

Bonaz B, Picq C, Sinniger V, Mayol JF, Clarencon D (**2013**) Vagus nerve stimulation: from epilepsy to the cholinergic anti-inflammatory pathway. Neurogastroenterol Motil 25: 208-221.

Brown DR, Timmermans JP (**2004**) Lessons from the porcine enteric nervous system. Neurogastroenterol Motil 16 (Suppl 1): 50-54.

Chang MM, Leeman SE (**1970**) Isolation of a sialogogic peptide from bovine hypothalamic tissue and its characterization as substance P. J Biol Chem 245: 4784-4790.

Couceyro P, Paquet M, Koylu E, Kuhar MJ, Smith Y (**1998**) Cocaine- and amphetamine-regulated transcript (CART) peptide immunoreactivity in myenteric plexus neurons of the rat ileum and co-localization with choline acetyltransferase. Synapse 30: 1-8.

Di Lorenzo M, Krantis A (**2002**) Nitric oxide synthase isoenzyme activities in a premature piglet model of necrotizing enterocolitis: effects of nitrergic manipulation. Pediatr Surg Int 18: 624-629.

Douglass J, McKinzie A A, Couceyro P (**1995**) PCR differential display identifies a rat brain mRNA that is transcriptionally regulated by cocaine and amphetamine. J Neurosci 15: 2471-2481.

Dun NJ, Dun SL, Kwok EH, Yang J, Chang J (**2000**) Cocaine- and amphetamine-regulated transcript-immunoreactivity in the rat sympatho-adrenal axis. Neurosci Lett 283: 97-100.

Ekblad E, Kuhar M, Wierup N, Sundler F (**2003**) Cocaine- and amphetamine-regulated transcript: distribution and function in rat gastrointestinal tract. Neurogastroenterol Motil 15: 545-557.

Ekblad E (**2006**) CART in the enteric nervous system. Peptides 27: 2024-2030.

Ellis LM, Mawe GM (**2003**) Distribution and chemical coding of cocaine-and amphetamine- regulated transcript peptide (CART)-immunoreactive neurons in the guinea pig bowel. Cell Tissue Res 312: 265-274.

Gonkowski S, Kamińska B, Burliński P, Kroll A, Całka J (**2009a**) The influence of drug – resistant ulcerative colitis on the number of cocaine- and amphetamine-regulated transcript peptide-like immunoreactive (CART-LI) mucosal nerve fibres of the descending colon in children. Przegląd Gastroenterologiczny 4: 147-151.

Gonkowski S, Burliński P, Skobowiat C, Majewski M, Arciszewski MB, Radziszewski P, Całka J (**2009b**) Distribution of cocaine- and amphetamine-regulated transcript-like immunoreactive (CART-LI) nerve structures in the porcine large intestine. Acta Vet Hung 57: 509-520.

Gonkowski S, Burliński P, Skobowiat C, Majewski M, Całka J (**2010**) Inflammation- and axotomy-induced changes in galanin-like immunoreactive (GAL-LI) nerve structures in the porcine descending colon. Acta Vet Hung 58: 91-103.

Gonkowski S, Burliński P, Szwajca P, Całka J (**2012**) Changes in cocaine- and amphetamine- regulated transcript - like immunoreactive (CART-LI) nerve structures of the porcine descending colon during proliferative enteropathy. Bull Vet Inst Pulawy 56: 199-203.

Grider JR, Murthy KS, Jin JG, Makhlouf GM (**1992**) Stimulation of nitric oxide from muscle cells by VIP: prejunctional enhancement of VIP release. Am J Physiol 262: G774-G778.

Gunnarsdóttir A, Wierup N, Larsson LT, Kuhar MJ, Ekblad E (**2007**) CART-peptide immunoreactivity in enteric nerves in patients with Hirschsprung's disease. Eur J Pediatr Surg 17: 184-189.

Harrington AM, Hutson JM, Southwell BR (**2010**) Cholinergic neurotransmission and muscarinic receptors in the enteric nervous system. Prog Histochem Cytochem 44: 173-202.

Holzer P (**1997**) Involvement of nitric oxide in the substance P-induced inhibition of intestinal peristalsis. Neuroreport 8: 2857-2860.

Janiuk I, Młynek K, Wysocki J (**2013**) Identification and location of the cocaine and amphetamine regulated transcript (CART) in the abomasum of cattle. Acta Histochem 115: 371-375.

Jensen PB, Kristensen P, Clausen JT, Judge ME, Hastrup S, Thim L, Wulff BS, Foged C, Jensen J, Holst JJ, Madsen OD (**1999**) The hypothalamic satiety peptide CART is expressed in anorectic and non-anorectic pancreatic islet tumors and in the normal islet of Langerhans. FEBS Lett 447: 139-143.

Kaleczyc J, Klimczuk M, Franke-Radowiecka A, Sienkiewicz W, Majewski M, Łakomy M (**2007**) The distribution and chemical coding of intramural neurons supplying the porcine stomach - the study on normal pigs and on animals suffering from swine dysentery. Anat Histol Embryol 36: 186-193.

Kasacka I, Piotrowska Z, Car H, Janiuk I, Lebkowski W (**2012**) Cocaine- and amphetamine-regulated transcript: identification and distribution in human gastrointestinal tract. J Biol Regul Homeost Agents 26: 419-428.

Koon HW, Pothoulakis C (**2006**) Immunomodulatory properties of substance P: the gastrointestinal system as a model. Ann N Y Acad Sci 1088: 23-40.

Kuhar MJ, Dall Vechia SE (**1999**) CART peptides: novel addiction- and feeding-related neuropeptides. Trends Neurosci 22: 316-320.

Lin Z, Sandgren K, Ekblad E (**2004**) Increased expression of nitric oxide synthase in cultured neurons from adult rat colonic submucous ganglia. Auton Neurosci 114: 29-38.

Luyer MD, De Haan JJ, Lubbers T, Greve JW, Buurman WA (**2013**) Parasympathetic stimulation via the vagus nerve prevents systemic organ dysfunction by abrogating gut injury and lymph toxicity in trauma and hemorrhagic shock. Shock 39: 460-461.

Okumura T, Yamada H, Motomura W, Kohgo Y (**2000**) Cocaine-amphetamine-regulated transcript (CART) acts in the central nervous system to inhibit gastric acid secretion via brain corticotropin-releasing factor system. Endocrinology 141: 2854-2860.

Olsson C, Holmgren S (**2001**) The control of gut motility. Comp Biochem Physiol A Mol Integr Physiol 128: 481--503.

Porcher C, Julé Y, Henry M (**2000**) A qualitative and quantitative study on the enkephalinergic innervation of the pig gastrointestinal tract. J Histochem Cytochem 48: 333-344.

Risold PY, Bernard-Franchi G, Collard C, Jacquemard C, La Roche A, Griffond B (**2006**) Ontogenic expression of CART-peptides in the central nervous system and the periphery: a possible neurotrophic role? Peptides 27: 1938-1941.

Sandgren K, Lin Z, Fex Svenningsen A, Ekblad E (**2003**) Vasoactive intestinal peptide and nitric oxide promote survival of adult rat myenteric neurons in culture. J Neurosci Res 72: 595-602.

Sayegh AI, Ritter RC (**2003**) Cholecystokinin activates specific enteric neurons in the rat small intestine. Peptides 24: 237-244.

Schemann M, Schaaf C, Mader M (**1995**) Neurochemical coding of enteric neurons in the guinea pig stomach. J Comp Neurol 353: 161-178.

Skobowiat C, Gonkowski S, Calka J (**2010**) Phenotyping of sympathetic chain ganglia (SChG) neurons in porcine colitis. J Vet Med Sci 72: 1269-1274.

Spiess J, Villarreal J, Vale W (**1981**) Isolation and sequence analysis of a somatostatin-like polypeptide from ovine hypothalamus. Biochemistry 20: 1982-1988.

Tebbe JJ, Ortmann E, Schumacher K, Monnikes H, Kobelt P, Arnold R, Schaffer M K-H (**2004**) Cocaine- and amphetamine-regulated transcript stimulates colonic motility via central CRF receptor activation and peripheral cholinergic pathways in fed conscious rats. Neurogastroenterol Motil 16: 489-496.

Verma N, Rettenmeier AW, Schmitz-Spanke S (**2011**) Recent advances in the use of Sus scrofa (pig) as a model system for proteomic studies. Proteomics 11: 776-793.

Wierup N, Gunnarsdóttir A, Ekblad E, Sundler F (**2007**) Characterisation of CART-containing neurons and cells in the porcine pancreas, gastro-intestinal tract, adrenal and thyroid glands. BMC Neurosci 8: 51.

Wojtkiewicz J, Gonkowski S, Bladowski M, Majewski M (**2012**) Characterization of cocaine- and amphetamine-regulated transcript-like immunoreactive (CART-LI) enteric neurons in the porcine small intestine. Acta Vet Hung 60: 371-381.

Zacharko-Siembida A, Arciszewski MB (**2014a**) Cocaine- and amphetamine-regulated transcript-like immunoreactivity (CART-LI) in intramural ganglia of porcine urinary bladder trigone. Med Weter 70: 549-598.

Zacharko-Siembida A, Arciszewski MB (**2014b**) Immunoreactivity to cocaine- and amphetamine- regulated transcript in the enteric nervous system of the pig and wild boar stomach. Anat Histol Embryol 43: 48-55.