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COCAINE- AND AMPHETAMINE-REGULATED TRANSCRIPT (CART) PEPTIDE IN MAMMALS GASTROINTESTINAL SYSTEM – A REVIEW*

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Abstract

Since its first description over 30 years ago, cocaine- and amphetamine-regulated transcript (CART) peptide has been the subject of many studies. Most of these investigations pertain to occurrence and functions of CART within the central nervous system, where this peptide first of all takes part in regulation of feeding, stress reactions, as well as neuroprotective and neuroregenerative processes. However, in recent years more and more studies concern the presence of CART in the gastrointestinal system. This peptide has been described both in stomach and intestine, as well as in other digestive organs such as pancreas or gallbladder. Particularly much information relates to distribution of CART in the enteric nervous system, which is located within the wall of digestive tract. Other studies have described this peptide in intestinal endocrinal cells. Moreover, it is known that CART can be present in various types of neuronal cells and may co-localize with different types of other neuronal active substances, which play roles of neuromediators and/or neuromodulators. On the other hand precise functions of CART in the gastrointestinal system still remain unknown. It is assumed that this peptide is involved in the regulation of gastrointestinal motility, intestinal blood flow, secretion of intestinal juice, somatostatin and/or insulin, as well as takes part in pathological processes within the gastrointestinal tract. The large number of recent studies concerning the above mentioned problems makes that knowledge about occurrence and functions of CART in the digestive system rather piecemeal and requires clarifying, which is the aim of the present article.

Key words: CART peptide, digestive system, enteric nervous system

Cocaine- and amphetamine-regulated transcript (CART) peptide has been described for the first time in the ovine hypothalamus in 1981 (Spiess et al., 1981) and the name of this substance derives from increasing amount of CART mRNA in the rat striatum after administration of cocaine and amphetamine (Douglass et al., 1995).

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From the time of discovery, CART has been described in various types of tissues including neuronal and endocrine cells. First of all, this peptide has been observed in the central nervous system, where particularly it concentrates in the hypothalamus (Douglass et al., 1995; Koylu et al., 1997). Previous studies have shown that CART also occurs in different parts of the peripheral nervous system, such as primary sensory neurons (Dun et al., 2000), as well as nerves supplying the adrenal and thyroid glands (Wierup et al., 2007). But especially many investigations in recent years have described CART in the digestive system, where it has been observed in both neuronal and endocrine cells in stomach, small and large intestine, pancreas and gallbladder. A great deal of information applying to CART in the digestive system makes this knowledge very chaotic. Therefore, the aim of this article is to systematize and review information concerning the occurrence and functions of CART in the gastrointestinal tract and organs connected with digestion and absorption of food.

CART structure

Cocaine- and amphetamine-regulated transcript (CART) peptide is derived from a proCART polypeptide. In rats there are two isoforms of proCART (Dylag et al., 2006), which, depending on the number of amino acids, are called proCART 1–89 and proCART 1–102 (Figure 1). Pro-peptides undergo post-translational changes with convertase enzymes and during these processes various biological active forms of CART peptides arise. The most important of them on grounds of the largest biological activity is CART 55–102 and CART 62–102 (Kuhar and Yoho, 1999). In human only one form of pro-CART (1-89) has been described and active peptides derived from it are called CART 42–89 and CART 49–89. A characteristic of the CART molecule, irrespective of isoform, is the presence of three disulfide bonds (Figure 1). It should be pointed out that CART is conserved in respect of evolutionary changes and identity of amino acids sequence between human and rat peptide is 95%. The only difference between CART 55–102 and CART 42–89 consists in replacement of isoleucine (55) in longer form by valine (42) in CART 42–89 (Douglass and Daoud, 1996; Dylag et al., 2006).

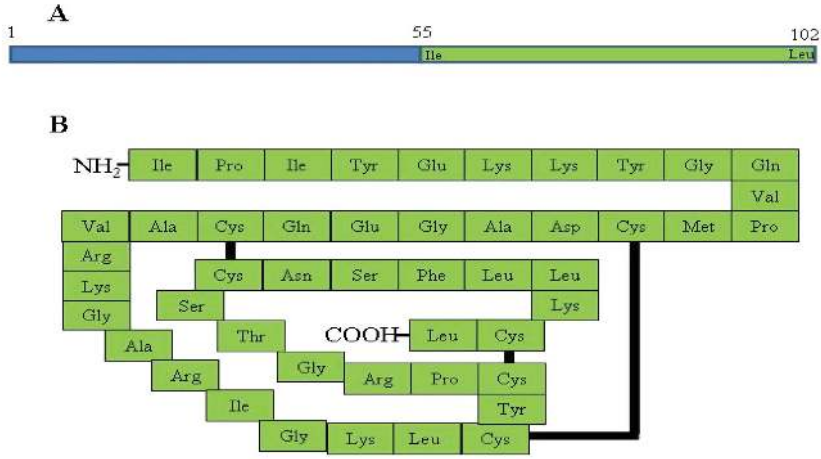

Figure 1. Scheme of the rat proCART 1–102 (A) and CART 55–102 (B)

CART in the enteric nervous system

Organization of the enteric nervous system

The enteric nervous system (ENS), which controls all functions of digestive tract, such as intestinal motility, secretion of enzymes and blood flow is located in the wall of esophagus, stomach and intestine and characterized by significant independence from the central nervous system. The structure of the ENS clearly depends on both the intestinal segment and mammal species (Figure 2). In small animals (rodents) it consists of two ganglionated plexuses (Figure 2 A): myenteric plexus located between longitudinal and circular muscle layers and submucous plexus – in the submucosa (Furness, 2000). In the intestine of large animals (for example pig) (Figure 2 B) submucosal plexus is divided into outer submucous plexus located near internal side of the circular muscle layer and inner submucous plexus – between the muscular mucosa and lamina propria (Balemba et al., 1998). However, in esophagus and stomach of large animals the ENS consists of two kinds of ganglia (like in small animals): myenteric ganglia, which are connected to each other by dense network (Timmermans et al., 1992; Furness, 2012) of nerves and create myenteric plexus and submucous ganglia, which do not form a plexus. Moreover in ruminants' forestomach only myenteric plexus has been observed (Münnich et al., 2008; Arciszewski et al., 2009). In human intestines (Figure 2 C) submucous plexus is divided into three parts: outer submucous plexus and inner submucous plexus (situated like in large animals), as well as intermediate plexus located between both mentioned above and containing only nerve fibers without neuronal cell bodies (Crowe et al., 1992; Ibba-Manneschi et al., 1995). Besides the above-mentioned plexuses the ENS of all mammal species also includes neuronal cells scattered in the mucosal and submucosal layers located in all segments of the digestive tract from esophagus to anus (Furness, 2000).

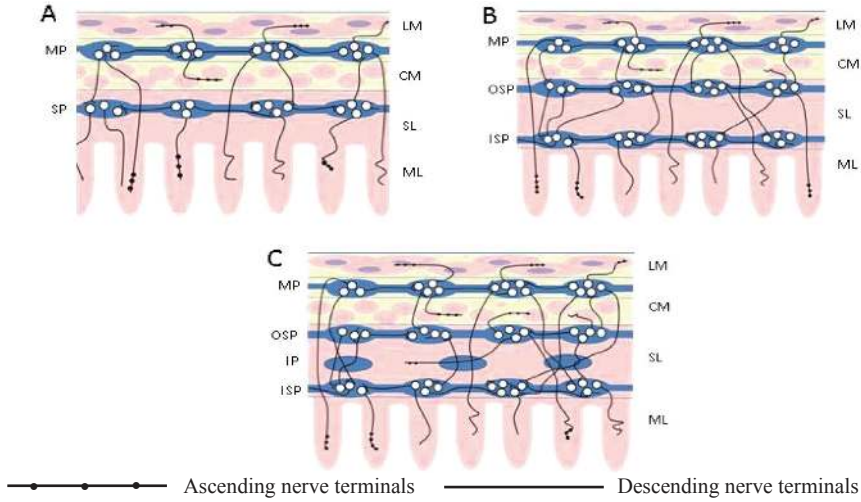

Figure 2. Scheme of the enteric nervous system in: A – rodents, B – big mammals (such as the pig), C – human. Elements of intestinal wall: LM – longitudinal muscle layer, CM – circular muscle layer, SL – submucosal layer. ML – mucosal layer. Elements of the enteric nervous system: MP – myenteric plexus, OSP – outer submucous plexus, IP – intermediate plexus, ISP – inner submucous plexus

The ENS consists of millions of neurons, which belong to several morphological and functional classes. One of the signs of extraordinary diversification and versatility of the ENS is multifariousness of neuronal active substances observed within enteric neurons (Furness, 2000). Until now, several such substances, which can play roles of neuromediators and/or neuromodulators, have been observed and still new types of them are discovered. One of such substances, which has been relatively recently described in the ENS is CART.

Occurrence of CART in the enteric nervous system

CART has been observed within the ENS of various segments of the digestive tract in both neuronal cells and nerve fibers located in mucosal and muscular layers. The percentage of CART-positive neurons and nerves clearly depends on intestinal fragment, "kind" of enteric plexus, as well as animal species. Because in 2006 the state of knowledge concerning CART in the ENS has been accurately described by Ekblad (2006), the present chapter mainly focuses on the review of investigations performed since 2007 unto this day.

Relatively the least is known about CART in the ENS of esophagus. Only one study (Kasacka et al., 2012 b) has described CART within muscular layer and mucosal plexus in this segment of the digestive tract of human. Contrary to esophagus, the knowledge about CART in the ENS of other segments of the digestive tract is more complete. The majority of studies on this area of knowledge in the last years have been performed on pigs. It should be pointed out that this fact is not accidental because the domestic pig becomes an important laboratory animal due to well-known similarities in anatomical, histological, biochemical and physiological characteristics to human organism (Verma et al., 2011). These similarities are especially visible in organization of the ENS (Brown and Timmermans, 2004). So, pig seems to be an optimal animal model of human ENS organization.

The relatively numerous population of CART-positive neurons has been observed in mucosal plexus of porcine stomach. The percentage of these neuronal cells fluctuates from about 40% (Zacharko-Siembida and Arciszewski, 2014) to over 50% (Bulc et al., 2015 a) of all myenteric neurons within gastric antrum. Fewer number of neurons immunoreactive to CART have been observed in gastric corpus and pylorus, and differences in the number of CART-positive cell bodies in particular authors are more visible. Namely, the percentage of myenteric neuronal cells ranged from about 18% (Zacharko-Siembida and Arciszewski, 2014; Rękawek et al., 2015) to over 30% (Bulc et al., 2015 a) in gastric corpus and from about 14% (Bulc et al., 2015 a) to over 30% (Zacharko-Siembida and Arciszewski, 2014) in the pylorus. CART-positive neurons have not been observed within submucous plexus in the porcine stomach (Wierup et al., 2007; Zacharko-Siembida and Arciszewski, 2014; Bulc et al., 2015 a; Rękawek et al., 2015). Similar distribution of CART-like immunoreactive enteric neurons has been observed in the stomach of human (Kasacka et al., 2012 b), wild boar (Zacharko-Siembida and Arciszewski, 2014) and rat (Kasacka and Piotrowska, 2012). In regard to nerve fibers located in the gastric wall, the majority of CART-positive nerves in all species studied has been observed in muscular layers and inside myenteric plexus (Kasacka et al., 2012 b; Kasacka and Piotrowska,

2012; Zacharko-Siembida and Arciszewski, 2014; Bulc et al., 2015 a), while only single fibers have been noted in mucosal layer and submucous plexus (Bulc et al., 2015 a).

Moreover, the presence of CART in neurons and nerve fibers has been also observed in forestomachs and stomach of sheep (Arciszewski et al., 2009), where neuronal cells immunoreactive to this peptide formed about 50% of all neurons in myenteric plexus within the rumen, reticulum, omasum and abomasum.

Similar distribution of CART-like immunoreactive enteric neuronal structures has been observed in small intestine. In all species studied the majority of CART--positive neurons are located in the myenteric plexus (Kasacka et al., 2012 b; Kasacka and Piotrowska, 2012; Wojtkiewicz et al., 2012). In pigs, where distribution of CART in the ENS of small intestine has been described most accurately (Wojtkiewicz et al., 2012) the percentage of neurons immunoreactive to CART in myenteric plexus fluctuated between about 10% in duodenum to over 20% in ileum. Clearly lesser number of CART-positive cells has been observed in outer submucous plexus (from about 3% in jejunum to over 10% in duodenum) and inner submucous plexus (single CART-positive neurons in jejunum and ileum and about 2% of all neuronal cells in outer submucous plexus of duodenum). CART in nerve fibers in all studied species has been mainly noted (like in the stomach) within muscular layers and inside myenteric plexus (Kasacka et al., 2012 b; Kasacka and Piotrowska, 2012; Wojtkiewicz et al., 2012), although such nerves have been also noted in the mucosal layer (Wojtkiewicz et al., 2012; Rychlik et al., 2015).

The most recent studies concerning distribution of CART in the ENS of large intestine have been also performed on human and pigs. In human large intestine the numerous population of CART-positive neurons has been observed mainly in the myenteric plexus from cecum to rectum (Gonkowski et al., 2009 a; Kasacka et al., 2012 b), and the large number of CART-immunoreactive nerves has been noted especially in cecum and descending colon (Gonkowski et al., 2013; Bulc et al., 2014, 2015 b). In large intestine of the pig, where data are more precise (Gonkowski et al., 2009 a) the number of CART-positive neuronal cells within myenteric plexus fluctuated from over 2% of all myenteric neurons in cecum to over 7% in centripetal turns of the proximal colon and rectum. In the outer submucous plexus the largest number of neuronal cells immunoreactive to CART has been noted in rectum (5%), and in the inner submucous plexus these values are rather equalized in all segments of the large intestine and fluctuated around 3% (Gonkowski et al., 2009 a).

Moreover, a dense meshwork of CART-like immunoreactive nerves has been observed in the muscular layer of all segments of the large intestine, not only in pigs (Gonkowski et al., 2009 a), but also in rats (Kasacka and Piotrowska, 2012). On the other hand such fibers have been rather sporadically observed in the mucosal layer of all species studied (Gonkowski et al., 2009 a, b; Kasacka et al., 2012 b; Rychlik et al., 2015). Recent data applying to occurrence of CART in the ENS of various species are presented in Table 1.

Table 1. Distribution of cocaine- and amphetamine-regulated transcript peptide in the enteric nervous system in mammals

Species / Fragment of the gastrointestinal tract	Human	Guinea pig	Pig	Wild boar	Sheep	Rat	References
Esophagus	+						Kasacka et al., 2012
Forestomachs/ stomach	+		+	+	+	+	Murphy et al., 2000; Ekblad et al., 2003; Wierup et al., 2007; Arciszewski et al., 2009; Kasacka et al., 2012; Zacharko-Siembida and Arciszewski, 2014
Duodenum	+	+	+			+	Murphy et al., 2000; Ekblad et al., 2003; Ellis and Mawe, 2003; Wierup et al., 2007; Kasacka et al., 2012 b; Wojtkiewicz et al., 2012
Jejunum	+		+			+	Murphy et al., 2000; Ekblad et al., 2003; Wierup et al., 2007; Kasacka et al., 2012 b; Wojtkiewicz et al., 2012
Ileum	+	+	+			+	Couceyro et al., 1998; Murphy et al., 2000; Ekblad et al., 2003; Ellis and Mawe, 2003; Kasacka et al., 2012 b; Wojtkiewicz et al., 2012
Cecum	+	+	+			+	Murphy et al., 2000; Ellis and Mawe, 2003; Wierup et al., 2007; Gonkowski et al., 2009 a; Kasacka et al., 2012 b; Bulc et al., 2014, 2015 b
Proximal colon		+	+			+	Murphy et al., 2000; Ekblad et al., 2003; Ellis and Mawe, 2003; Gonkowski et al., 2009 a; Kasacka et al., 2012 b; Palus and Rytel, 2013
Distal colon	+	+	+			+	Murphy et al., 2000; Ellis and Mawe, 2003; Gonkowski et al., 2009 a, b, 2013
Rectum			+			+	Murphy et al., 2000; Gonkowski et al., 2009 a

Co-localization of CART with other active substances in the ENS

It is well known that one neuron can contain few or even several active substances, which may play roles of neuromediators and/or neuromodulators. Co-localization of various substances are also observed in the enteric nervous system, where each functional class of neuronal cells has unique combinations of active neurochemical

factors (Furness, 2000), also known as "chemical coding". Substances, which are present in the same neuron also play similar roles and take part in alike regulatory processes, and investigations on co-localization of neuronal factors is one of methods to determine their exact functions. On the other hand, until now functions of CART within the digestive system are not fully explained. Thus, relatively many recent studies apply to co-localization of CART with other neuronal factors in neurons of the ENS.

The co-localization of CART with various other neuronal active substances has been observed in enteric neurons. One of these substances is vasoactive intestinal polypeptide (VIP), which is known as important inhibitor of intestinal motility and gastric acid secretion, as well as substance which regulates immunological processes, neuroprotective mechanisms and the blood flow in the wall of the digestive tract (Van Geldre and Lefebvre, 2004; Arciszewski and Ekblad, 2005). Co-localization of CART and VIP has been observed in all segments of the digestive tract of various species, both in neuronal cells (Arciszewski et al., 2009; Wojtkiewicz et al., 2012; Palus and Rytel, 2013) and nerve fibers (Wierup et al., 2007; Arciszewski et al., 2009; Gonkowski et al., 2013; Bulc et al., 2015 b). The level of coexistence of these substances in the same neuronal structures has been very high and constituted even 90% of all CART-positive structures (Wierup et al., 2007). Interestingly, the co-localization of CART with pituitary adenylate cyclase-activating polypeptide (PACAP), which is similar in 68% to VIP and also (like VIP) takes part in relaxation of intestinal muscles (Läuff et al., 1999), is lower (Gonkowski et al., 2013).

Other substances, which also often occur with CART in the same neuronal enteric structures are choline acetyltransferase (ChAT) (or vesicular acetylcholine transporter – VAChT), nitric oxide synthase (NOS), galanin (GAL) and substance P (SP).

ChAT and VAChT are markers of cholinergic neurons, and acetylcholine is the main neuromediator of the ENS (Furness, 2000), which takes part in the contraction of intestinal muscles and stimulates the secretion from intestinal glands (McConalogue and Furness, 1994; Ogura et al., 2007; De Jonge, 2013). The high level of co-localization of CART and ChAT (or VAChT) has been noted in enteric neurons and nerve fibers in duodenum, ileum, caecum and colon of various mammal species, including human (Couceyro et al., 1998; Ellis and Mawe, 2003; Gonkowski et al., 2013).

In turn, NOS is a marker of neurons using nitric oxide as a gaseous neuromediator – nitric oxide, which primarily has inhibitory effects on the intestinal motility (Sarna et al., 1993; Schleiffer and Raul, 1997). Co-localization of CART and NOS has been observed in myenteric plexus and muscular nerve fibers of various parts of the digestive tract (Ellis and Mawe, 2003; Ekblad et al., 2003; Wierup et al., 2007; Wojtkiewicz et al., 2012; Gonkowski et al., 2013; Bulc et al., 2015 b), while NOS has not been studied within CART-positive neuronal structures in mucosal and submucosal layers (Ekblad et al., 2003).

Galanin, which functions in the digestive tract and clearly depends on both the segment of intestine and animal species studied (Fox-Threlkeld et al., 1991; Sar-

nelli et al., 2004) has been also often observed in CART-positive structures of the ENS, especially in human and pig (Gonkowski et al., 2013; Zacharko-Siembida and Arciszewski, 2014; Bulc et al., 2015 b). Similar observations concern substance P, which takes part in the regulation of intestinal motility, secretion and transmission of pain stimuli (Thor et al., 1982; Gonkowski, 2013). Co-localization of CART and SP has been studied in the ENS of forestomachs of sheep (Arciszewski et al., 2009), as well as in stomach (Zacharko-Siembida and Arciszewski, 2014), small (Wojtkiewicz et al., 2012) and large (Gonkowski et al., 2013; Bulc et al., 2014) intestine of various mammal species, including human.

Apart from the substances mentioned above, also other neuronal active factors such as calcitonin gene related peptide (Bulc et al., 2014), somatostatin (Arciszewski et al., 2009; Wojtkiewicz et al., 2012), neuropeptide Y (Arciszewski et al., 2009), leu-enkephalin (Bulc et al., 2014) and/or neurokinin A (Bulc et al., 2014) have been observed in CART-positive structures within the ENS, but it should be pointed out that the level of co-localization of CART and these factors has been rather low. The recent knowledge concerning the co-localization of CART with other active substances in the ENS is presented in Table 2.

Plasticity of CART-positive enteric neurons

The term "plasticity of the nervous system" means each adaptive modification of neuronal cells under various stimuli. It is well established that this phenomenon often occurs in the ENS. Namely, enteric neurons can undergo structural, functional and/or neurochemical changes in response to both physiological and pathological factors. Structural changes of the ENS mainly consist in modifications of number and size of enteric neurons. These changes have been observed during physiological growth and development of living organism, as well as during some pathological states such as Crohn's disease and ulcerative colitis, where hypertrophy (the growth of size) and hyperplasia (the growth of number) of enteric neurons were noted (Bassotti et al., 2009), as well as during Hirschsprung's disease, in which the number of enteric neurons are extremely lesser (McKeown et al., 2013). In turn, functional changes of the ENS are observed mainly during various types of inflammatory processes and consist in disturbances in intestinal motility and excretive functions of the digestive tract (Vasina et al., 2006), which may manifest by nausea, vomiting and diarrhea. Nonetheless, the main manifestation of plasticity of the enteric nervous system is neurochemical changes of neurons. Previous studies show that enteric neurons may modify expression of active substances (mainly neuromediators and/or neuromodulators) under various physiological and pathological stimuli (Gonkowski et al., 2003, Vasina et al., 2006; Gonkowski et al., 2010; Rivera et al., 2011), and the character of these changes depends not only on the type of acting factor, but also on fragment of digestive tract and species of experimental animals (Vasina et al., 2006; Kasparek et al., 2007; Gonkowski and Całka, 2012). Moreover, changes in chemical coding of enteric neurons may be first, subclinical symptoms of intestinal diseases or influence of toxins in food (Gonkowski et al., 2015).

Table 2. Co-localization of cocaine- and amphetamine-regulated transcript peptide with other neuromediators in mammals gastrointestinal tract. (VIP – vasoactive intestinal peptide; PACAP – pituitary adenylate cyclase-activating polypeptide; ChAT – choline acetylotransferase; VAChT – vesicular acetylocholine transporter; nNOS – neuronal isoform of nitric oxide synthase; SP – substance P; L–ENK – leu 5 enkephalin; NKA – neurokinin A; SOM – somatostatin; NPY – neuropeptide Y; CGRP – calcitonin gene related peptide)

Neuromediator / Fragment of the gastrointestinal tract	VIP	PACAP	ChAT/ VAChT	nNOS	GAL	SP	L-ENK	NKA	SOM	NPY	CGRP	References
Esophagus												
Forestomachs/ /stomach	+			+	+	+			+	+	+	Ekblad et al., 2003; Wierup et al., 2007; Arciszewski et al., 2009; Zacharko-Siembida and Arciszewski, 2014
Duodenum	+			+		+			+		+	Ekblad et al., 2003; Wierup et al., 2007; Wojtkiewicz et al., 2012
Jejunum	+			+		+			+		+	Ekblad et al., 2003; Wierup et al., 2007; Wojtkiewicz et al., 2012
Ileum	+		+	+		+			+			Couceyro et al., 1998; Ekblad et al., 2003; Ellis and Mawe, 2003; Wojtkiewicz et al., 2012
Cecum	+			+	+	+	+	+			+	Wierup et al., 2007; Bulc et al., 2014; Bulc et al., 2015
Proximal colon	+			+								Ekblad et al., 2003; Palus and Rytel, 2013
Distal colon	+	+	+	+	+	+						Ekblad et al., 2003; Gonkowski et al., 2013
Rectum												

In spite of the fact that neurochemical changes of the ENS under various stimuli are described in numerous studies, the knowledge about plasticity of CART-positive neurons is rather scanty. Previous investigations show that expression of CART in the enteric nervous structures can undergo changes under various factors and their character depends on the type of acting factor, part of the ENS, fragment of the digestive tract and animal species. Namely, it has been shown that the number of CART-positive enteric nervous structures may increase during ulcerative colitis in human (Gonkowski et al., 2009 b), hypertension in rats (Kasacka and Piotrowska, 2012), inflammatory bowel disease in dogs (Rychlik et al., 2015) and culture of ENS cell *in vitro* (Ekblad, 2006), as well as after intoxication of zearalenone (Gonkowski et al., 2015). In contrast, the decrease of CART-like immunoreactive enteric nervous structures has been observed during Hirschsprung's disease (Gunnarsdóttir et al., 2007) and diabetes mellitus (Bulc et al., 2015 a). In turn, the experiments on experimental-induced inflammation and axotomy, show that changes in expression of CART in the ENS depend on the type of intramural ganglia (Burliński, 2012). Namely, in the mentioned study the decrease of CART-positive neurons has been observed in myenteric and outer submucous ganglia during inflammation and after axotomy, whereas the expression of CART in neurons of inner submucous ganglia and intramural nerve fibers was higher. Similar changes have been observed in studies on porcine proliferative enteropathy (Gonkowski et al., 2012 a). Moreover, it is known that some pathological factors (such as inflammation and axotomy) not only change the number of CART-positive enteric neurons, but also the degree of co-localization of this peptide with other active substances (Burliński et al., 2014).

CART in extrinsic innervation of the digestive tract

Despite significant autonomy of the ENS, functions of the digestive tract are also controlled by extrinsic innervation. This innervation is divided into three parts (Figure 3): afferent neurons, which relay information from the stomach and gut to the central nervous system, as well as efferent sympathetic and parasympathetic neuronal cells, which conduct impulses from the CNS to the digestive tract. Experiments using various types of retrograde neuronal tracers have shown that localization of neuronal cell bodies supplying the particular parts of the digestive tract is various. Afferent neurons supplying the GI tract are located in two sensory ganglia of vagal nerve (jugular and nodose ganglia), nucleus of the solitary tract, as well as in dorsal root ganglia (DRG) (Elfvin and Lindh, 1982). It is known that neurons in DRG of neuromers Th7-Th11 supply the stomach, Th7-12 – duodenum, L1-5 – jejunum and ileum, and S1 – colon (Su et al., 1987; Chiocchetti et al., 2006; Tan et al., 2008; Li et al., 2013).

Efferent parasympathetic neuronal cells supplying significant part of the digestive tract (from esophagus to proximal colon) are situated first of all in dorsal vagal motor nucleus (Won et al., 1998; Gańko and Całka, 2014 a, b). Only slight number of neurons innervating the stomach and gut have been noted in nucleus ambiguous (Won et al., 1998; Lam et al., 2009). Distal parts of the GI tract are supplied by neurons located in parasympathetic nuclei of sacral spinal cord (Million et al., 2000).

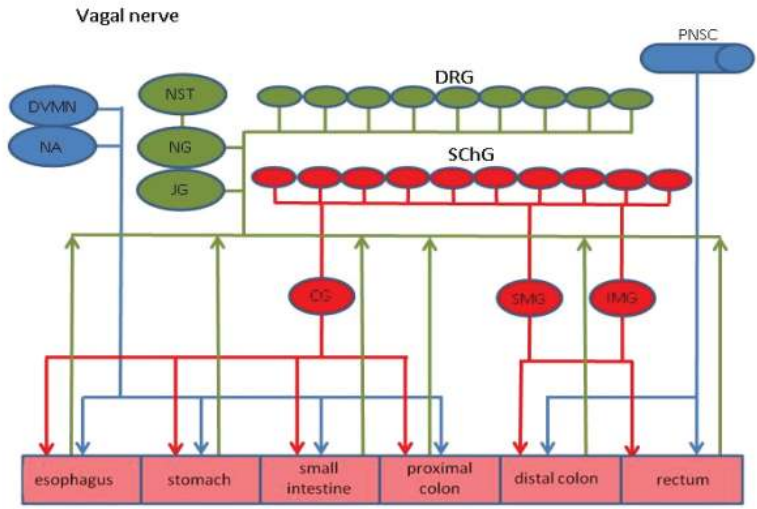

Figure 3. Scheme of extrinsic innervation of the gastrointesinal tract: green – afferent neurons, blue – parasympathetic neurons, red – sympathetic neurons. DVMN – dorsal vagal motor nucleus; NST – nucleus of the solitary tract; NA – nucleus ambiguus; NG – nodose ganglion; JG – jugular ganglion; DRG – dorsal root ganglia; PNSC – parasympathetic nuclei of the spinal cord; SChG – ganglia of the sympathetic chain; CG – celiac ganglion; SMG – superior mesenteric ganglion; IMG – inferior mesenteric ganglion

The localization of efferent sympathetic neurons supplying the GI tract also depends on the part of innervated fragment of gut. The significant part of the digestive tract (from esophagus to proximal colon) is innervated by neuronal cells located in celiac ganglia (Quinson et al., 2001). In turn, cell bodies situated in superior and inferior mesenteric ganglia provide their processes to distal colon and rectum (Kreulen and Szurszewski, 1979; Quinson et al., 2001; Wojtkiewicz et al., 2013). Moreover, it is known that some neurons supplying the digestive tract are situated in the sympathetic chain (Skobowiat et al., 2011).

In spite of the fact that CART has been noted in all above mentioned neuronal ganglia and nuclei, which can be a source of extrinsic innervation of the digestive system (Smith et al., 1997; Dun et al., 2001; Lam et al., 2009; Zacharko-Siembida et al., 2014; Palus and Całka, 2016), the knowledge about the presence of this peptide in neuronal cells really supplying the gastrointestinal tract (labelled by neuro-nal retrograde tracing) is very slight and, in fact, limited to three studies. Namely, CART has been noted in relatively large percentage of afferent parasympathetic cells innervating the stomach and duodenum, which are placed in rat nodose ganglia (Zheng et al., 2002). In this experiment CART has been observed in 17% of neurons with projections to the stomach and 41% to the duodenum. On the other hand, investigations on porcine dorsal vagal motor nucleus have not shown the presence of CART in neurons supplying the stomach (Gańko and Całka, 2014 a, b). Nevertheless, the participation of CART in extrinsic intestinal innervation is not excluded and further investigations are needed based on methods of retrograde neuronal tracing.

CART in the enteroendocrine cells of the digestive tract

Enteroendocrine cells located in the stomach, intestine and pancreas are the most numerous endocrine cells in living organism and form the enteric endocrine system (Ahlman and Nilsson, 2001; Gonkowski et al., 2012 b). These cells excrete various gastrointestinal hormones, which may be released to bloodstream or can play roles of the local messengers and, together with the enteric nervous system, are involved in the regulation of all gastrointestinal functions (Latorre et al., 2015). To date over one hundred bioactive peptides have been observed within the enteric endocrine system. One of them is CART.

It should be pointed out that knowledge about CART in intestinal endocrine cells is fragmentary and limited to a few species. Namely, the presence of CART-positive enteroendocrine cells has been noted in the stomach, duodenum, jejunum and ileum of human and rat, and the largest population of these cells has been observed in the mucosal layer of pylorus and duodenum (Ekblad et al., 2003; Kasacka et al., 2012 b; Kasacka and Piotrowska, 2012). Moreover, small population of CART-positive endocrine cells have been also observed in the abomasum of the sheep (Arciszewski et al., 2009). On the other hand, enteroendocrine cells immunopositive to CART have not been observed both in the large intestine of above mentioned species and in all (hitherto studied) parts of the mouse and porcine digestive tract (Wierup et al., 2007). Thus, the above mentioned data suggest that the degree of CART expression in enteroendocrine cells clearly depends on the part of the digestive tract and mammal species.

CART in the accessory digestive organs

Of the accessory organs of digestion, such as salivary glands, liver, pancreas and gallbladder, until now CART has been observed only in the last two mentioned above.

In the pancreas CART has been noted both within endocrine cells and in intrapancreatic nervous structures, and the place of occurrence of this peptide clearly depends on the age and species of animals studied. Namely, in immature rats CART has been shown within islets of Langerhans, in delta cells which are responsible for the production of somatostatin, as well as in nerve fibers and neurons localized in intra-pancreatic ganglia (Jensen et al., 1999; Wierup et al., 2004). In turn, in adult animals of some species (mice, rats, pigs) CART has been observed only within pancreatic nervous structures (Wierup et al., 2004, 2007; Moffett et al., 2006). These results strongly suggest that CART may be involved in processes connected with the pancreas growth and development during ontogenesis.

In other mammalian species, such as human or ruminants, CART in adult pancreas has been detected in both endocrine delta cells and nervous structures (Wierup et al., 2004, 2007; Wierup and Sundler, 2006). In the case of ruminants, CART occurs first of all in neuronal cells (about half of the population of neurons in pancreatic ganglia contain CART), whereas CART-positive endocrine cells are not numerous and localized in peripheral part of islets of Langerhans (Arciszewski et al., 2008; Janiuk and Młynek, 2015). In human, by contrast, endocrine cells immunopositive to CART are very numerous (Kasacka et al., 2012 a).

Information about CART in the gallbladder is very scanty and limited to one study (Ellis and Mawe, 2003), where this peptide has been noted in intramural neuronal structures of the gallbladder of guinea pig.

Functions of CART in the digestive system

In spite of the fact that CART has been described for the first time over thirty years ago, functions of this peptide remain not fully explained. Relatively most information concerns participation of this peptide in regulatory processes of food intake. Namely, CART is known as brain anorectic substance and important inhibitor of appetite, which can participate in the control of body weight (Kristensen et al., 1998; Vrang et al., 1999; Maletínská et al., 2008). Mechanism of the above mentioned function of CART is not fully explained, but probably it is connected with action of leptin, which regulates CART mRNA synthesis (Kristensen et al., 1998; Fekete and Lechan, 2006) and which is a hormone inhibiting the food intake (Friedman and Halaas, 1998). Other studies have shown that CART may participate in processes connected with stress, anxiety and reward responses (Jaworski and Jones, 2006; Vicentic and Jones, 2007; Balkan et al., 2012; Yoon et al., 2014).

It should be pointed out that the knowledge about functions of CART within the digestive tract is very scanty. Admittedly, it is known that this peptide reduces gastric acid secretion (Okumura et al., 2000), inhibits gastric emptying (Smedh and Moran, 2003) and stimulates colonic motility (Tebbe et al., 2004), but the exact mechanisms of these actions is unknown. Due to the fact that administration of CART to cell cultures does not affect the function of intestinal tissue (Tebbe et al., 2004), the above mentioned processes are probably regulated via the central nervous system.

It is assumed that some functions of CART within the digestive tract are similar to these which CART plays in other parts of the nervous system. These functions are the involvement in the development of the nervous system (Risold et al., 2006), neurotrophic and neuroprotective effects (Wu et al., 2006; Bharne et al., 2013), regenerative processes (Luo et al., 2013), as well as the role of endogenous antioxidant (Mao et al., 2012). Previous studies on the ENS, where changes of CART expression in neurons and nerve fibers have been noted under various pathological factors (Ekblad, 2006; Gonkowski et al., 2009 b, 2015; Kasacka and Piotrowska, 2012; Rychlik et al., 2015) seem to support above mentioned functions of this peptide in the digestive tract. Moreover, the localization of CART mainly in myenteric plexus of the ENS and intramuscular nerve fibers (Ekblad et al., 2003; Ellis and Mawe, 2003; Arciszewski et al., 2009; Gonkowski et al., 2009 a; Kasacka et al., 2012 b) suggests that first of all this peptide can be involved in regulatory processes of intestinal motility.

In the pancreas CART regulates the functions of pancreatic islets of Langerhans. Especially it influences the endocrine activity of beta (responsible for insulin secretion) and delta cells, which excrete somatostatin (Wierup and Sundler, 2006; Arciszewski et al., 2008; Kasacka et al., 2012 a). Moreover, CART takes part in the regulation of pancreatic blood flow (Arciszewski et al., 2008; Janiuk and Młynek, 2015) and neuroprotective processes within pancreatic nervous structures (Wierup et al., 2004).

Conclusion

In light of previous studies CART seems to be a very important regulatory factor within nervous and endocrine systems, which is also widespread within digestive organs. Till now it has been noted in the enteric nervous system of various parts of the gastrointestinal tract, nervous structures of gallbladder and pancreas, as well as in the enteric endocrine system. In spite of many experiments concerning the distribution of CART in the digestive organs, exact functions of this peptide in the stomach, intestine and pancreas remain unknown. It seems to be that CART first of all is involved in regulatory processes of intestinal motility and secretory activity, as well as takes part in neuroprotective and adaptive reactions under various pathological stimuli. Nevertheless, further studies are needed to elucidate exact physiological and pathological functions of CART within the digestive system.

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