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Dopa decarboxylase and angiotensin converting enzyme 2 immunoexpression in the gastrointestinal tract of control female Wistar rats — a pilot study

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Abstract: Studies have shown important interactions between the local renin–angiotensin and monoaminergic systems in physiology and pathophysiology. Yet the understanding of such interactions in the gastrointestinal (GI) tract and GI-associated diseases is the least understood. Thus, the aim of our study was to characterize the expression pattern of DDC and ACE2 along the GI tract (duodenum, jejunum, ileum, and colon) of control female Wistar rats.

Keywords: angiotensin-converting enzyme 2, aromatic L-amino acid decarboxylase, ACE2, DDC, Wistar rats.

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Introduction

Studies have shown important interactions between the local renin–angiotensin and monoaminergic (especially dopaminergic) systems in physiology and pathophysiology [1, 2]. Yet the understanding of such interactions in the gastrointestinal (GI) tract and GI-associated diseases is the least understood [3]. Renin-angiotensin and dopaminergic systems were reported to especially

influence on intestinal Na+/K+ ATPase activity [4–10]. Transcriptomic and immunohistochemical analysis confirmed the presence of angiotensin-converting enzyme 2 (ACE2; EC 3.4.17.23) and aromatic L-amino acid decarboxylase (AADC, also known as DOPA decarboxylase, DDC; EC 4.1.1.28) in SARS-CoV2-infected enterocytes [11], for example. AADC catalyzes several different decarboxylation reactions, including the decarboxylation of L-DOPA (l-3,4-dihydroxyphenylalanine) to dopamine [12]. Immunohistochemical studies have revealed that AADC is expressed in various neuronal cell types such as serotonergic and catecholaminergic neurons in the human brain [13], yet data with regard to GI remains limited. ACE2 is an enzyme that can be found either attached to the membrane of cells — mACE2 (enterocytes of duodenum and small intestine, for example) or in a soluble form (sACE2), and both are integral parts of the renin–angiotensin–aldosterone system [14]. Primarily, sACE2 cleaves the carboxyl-terminal amino acid phenylalanine from angiotensin II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) and hydrolyses it into angiotensin (1-7) (H-Asp-Arg-Val-Tyr-Ile-His-Pro-OH). sACE2 can cleave other peptides, including [des-Arg9]-bradykinin, apelin, neurotensin, dynorphin A, and ghrelin [15]. Furthermore, mACE2 also regulates the membrane trafficking of the neutral amino acid transporter SLC6A19 [16, 17].

ACE2 gained a lot of attention in the context of SARS-Co-2 pandemic [18]. So far, studies have shown important interactions between the local renin–angiotensin and dopaminergic systems in cardio-vascular diseases, neurodegeneration or inflammation [1–2], yet the understanding of such co-localization and functional interaction in the GI tract remains incomplete. Thus, the aim of this study was to characterize the expression pattern of DDC and ACE2 along the GI tract (duodenum, jejunum, ileum, and colon) of control female Wistar rats.

Material and Methods

Female control Wistar rats (n = 6; 190 g ± 20 g; Jagiellonian University Medical College Animal Laboratory, Kraków, Poland) were fed with standard dry chow: protein 25%, fat 8%, carbohydrates 67%, metabolizable energy 2.86 kcal/g (Labofeed B, Kcynia, Poland) and tap water was available ad libitum. The experiment was carried out in accordance with the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. After the period of acclimatization under controlled conditions, control rats were euthanized and tissue specimens from duodenum, jejunum, ileum, and colon were collected. Fresh specimens were rinsed with PBS (pH = 7.4), fixed in paraformaldehyde, routinely processed, embedded in paraffin (Modular Tissue Embedding Center EC 500, Especialidades Médicas MYR), cut into 5 μm thick sections (Microm HM 330 Microtome), and placed on slides with increased adhesion (Super Frost Plus, Menzel, Thermo Scientific). Hematoxylin–eosin staining was used to assess gross tissue organization. For immunolabelling, DOPA decarboxylase mouse monoclonal antibody (Invitrogen #CL2962; dilution 1:1000) and ACE2 rabbit monoclonal antibody (Invitrogen #SN0754; dilution 1:500) together with appropriate secondary antibodies (Alexa Fluor 488 and 594, Jackson Immunoresearch; dilution 1:1000) were used. Microscopic slides were examined using an Olympus BX43 epifluorescence microscope equipped with DP74 camera and cellSens Olympus software. The qualitative analysis of immunolabelling was provided in 20 consecutive fields of vision with 400× OR 600× magnification.

Results

Hematoxylin–eosin staining reveled typical architecture of small and large intestine of control rats. (Fig. 1). ACE2-immunoreactivity was not limited to small intestinal enterocytes but was present in all layers of the GI wall (Fig. 2–5). In the small intestine, the highest immunoreactivity of ACE2, apart from enterocytes, was noted in endothelial cells of the arterial and venous vessels and in the nerve plexuses. ACE-2 immunoreactivity was almost absent in the colonic mucosal layer. DDC immunoreacitity was expressed in different types of cells in all analyzed parts and layers of the GI tract (Fig. 2–5). Double immunofluorescence revealed significant co-localization of DDC and ACE2 across the GI wall (Fig. 2–5). DDC and ACE2 immunoreactivity was abundantly present in the small intestinal mucosa, submucosa, and both enteric plexuses. In the colon, their expression was predominant within submucosal and myenteric plexuses.

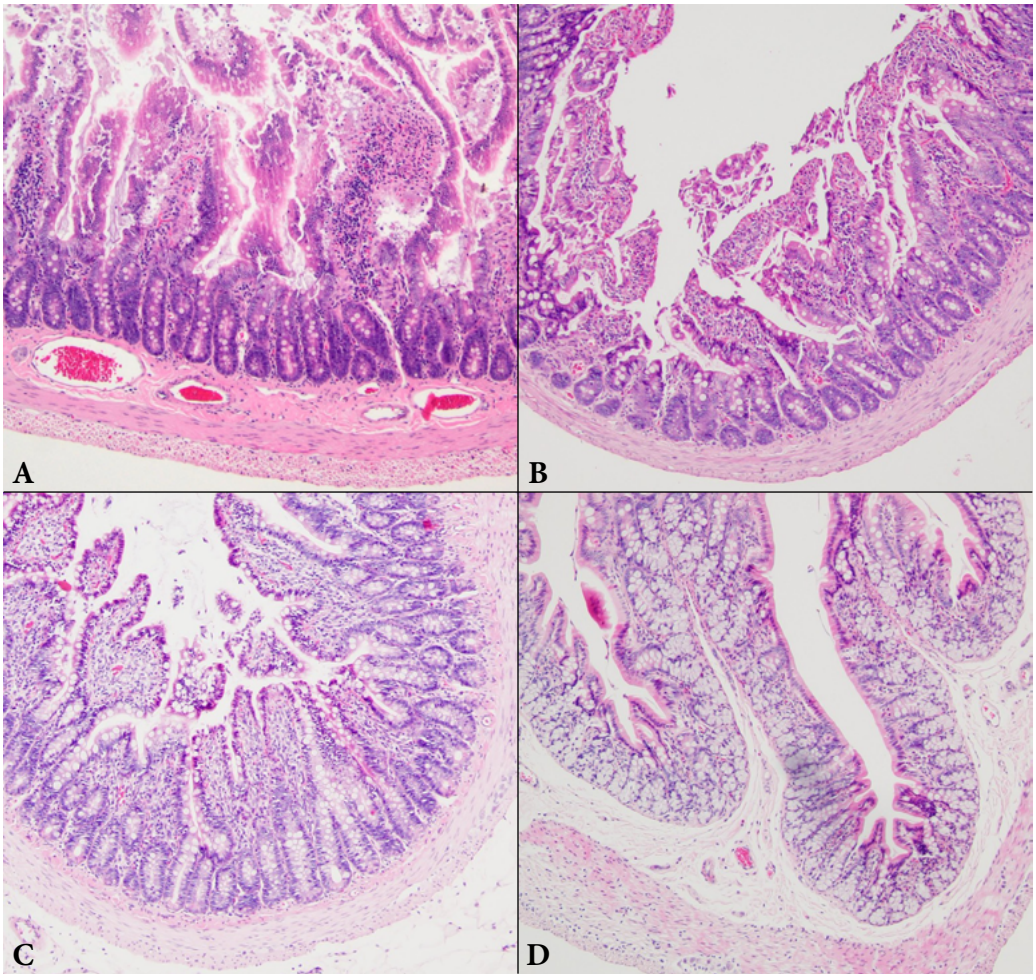

Fig. 1. Intestinal cross-sections stained with H&E: A) duodenum; B) jejunum; C) ileum; D) colon; magnification ×20 (A) or ×10 (B, C, D) Olympus DP74.

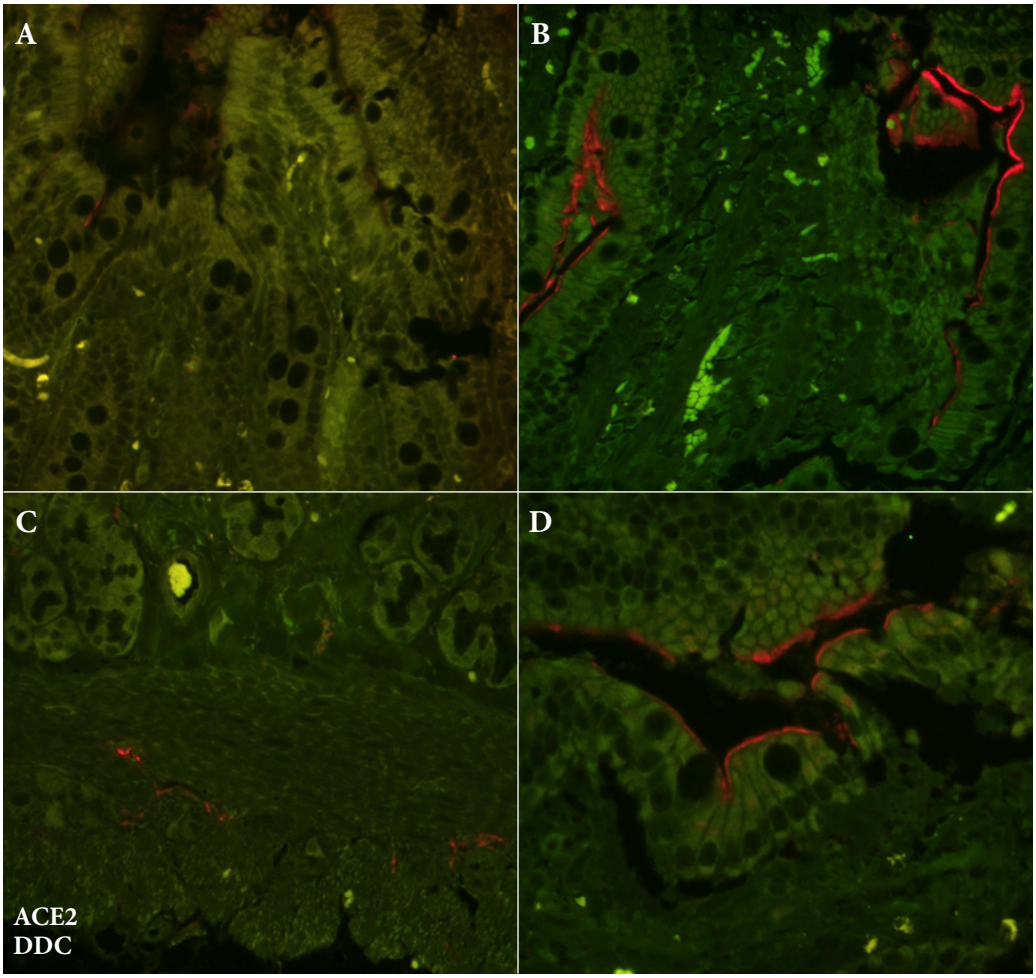

Fig. 2. Representative photomicrographs of duodenal cross-sections double-stained for ACE2 and DDC; magnification ×40 (A, B, C) or ×60 (D), Olympus DP74.

Discussion

ACE2 immunoexpression is well described in the context of gut enterocytes. SARS-CoV-2 pandemic immensely increased attention towards ACE2 and its multifunctional role in health and disease due to the fact that ACE2 is a specific functional receptor for the coronavirus [18]. The ACE2 protein also plays distinct biological roles independent of its well-described enzymic activity (being a metalloprotease operating through a zinc-dependent catalytic mechanism) as it appears to be a chimera composed of an ACE-like domain fused to a collectrin-like domain. ACE2 regulates transport of intestinal neutral amino acid transporters of the B0AT1 family to the plasma membrane and modulates intestinal inflammatory response, hence have the ability to influence on the gut microbiome and the gut-brain axis [17]. As demonstrated by our immunostaining results,

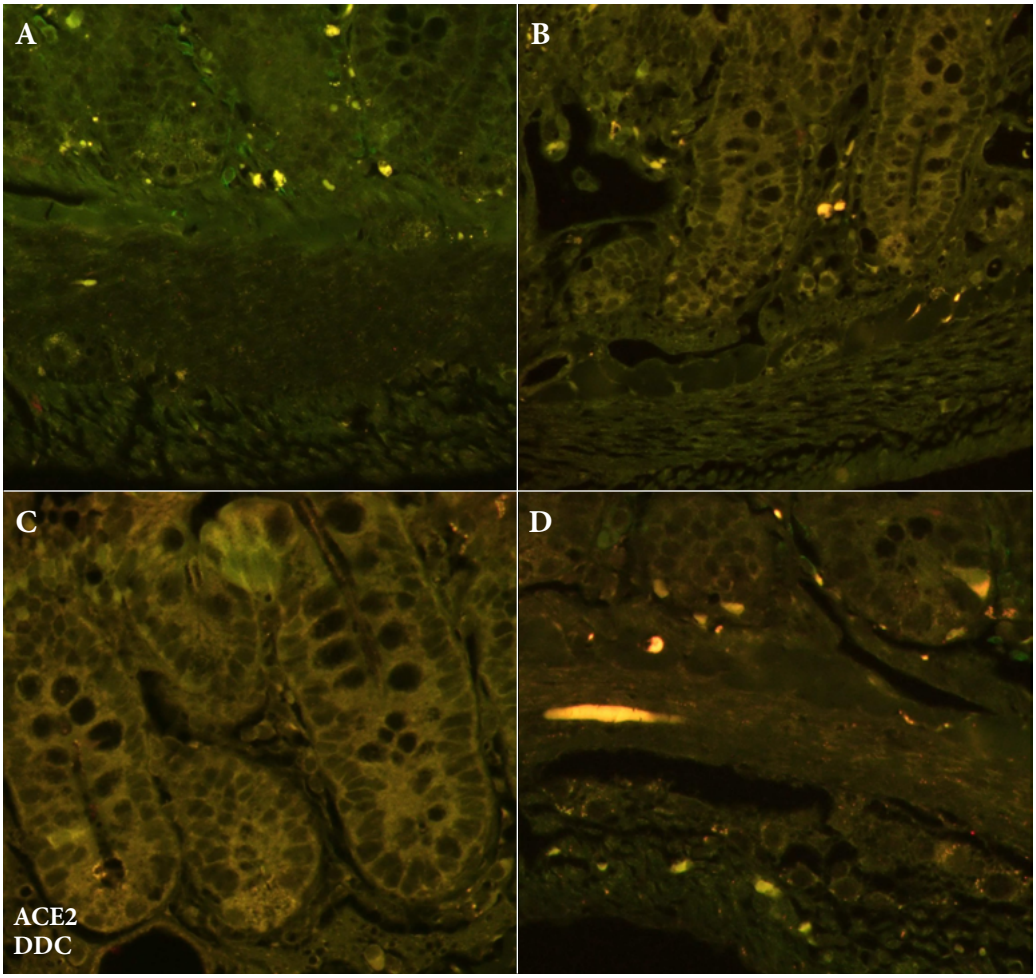

Fig. 3. Representative photomicrographs of jejunal cross-sections double-stained for ACE2 and DDC; magnification ×40 (A, B, C) or ×60 (D), Olympus DP74.

ACE2 is also present within the enteric plexus, however its role there remains unclear. However, it was already reported that ACE2 was expressed by enteric neurons and glial cells, and was suggested to serve as alternative entry routes of neuroinvasion by SARS-CoV-2 [19, 20]. In the central nervous system, ACE2 expression is not restricted to a single type of neural cell or non-neuronal cells such as astrocytes, oligodendrocytes, microglia, and endothelial cells. In fact, ACE2 may regulate neurotransmitter release, synaptic plasticity, neuroinflammation and maintainance of extracellular matrix homeostasis, may affect myelination and nerve conduction velocity as well as contribute to the integrity of the blood brain barrier [21]. Thus, ACE2 could also play an important role in the enteric nervous system.

The synthesis of multiple amine neurotransmitters, such as dopamine, norepinephrine, serotonin, and trace amines, relies partly on DOPA decarboxylase. Altogether, tyrosine hydroxylate

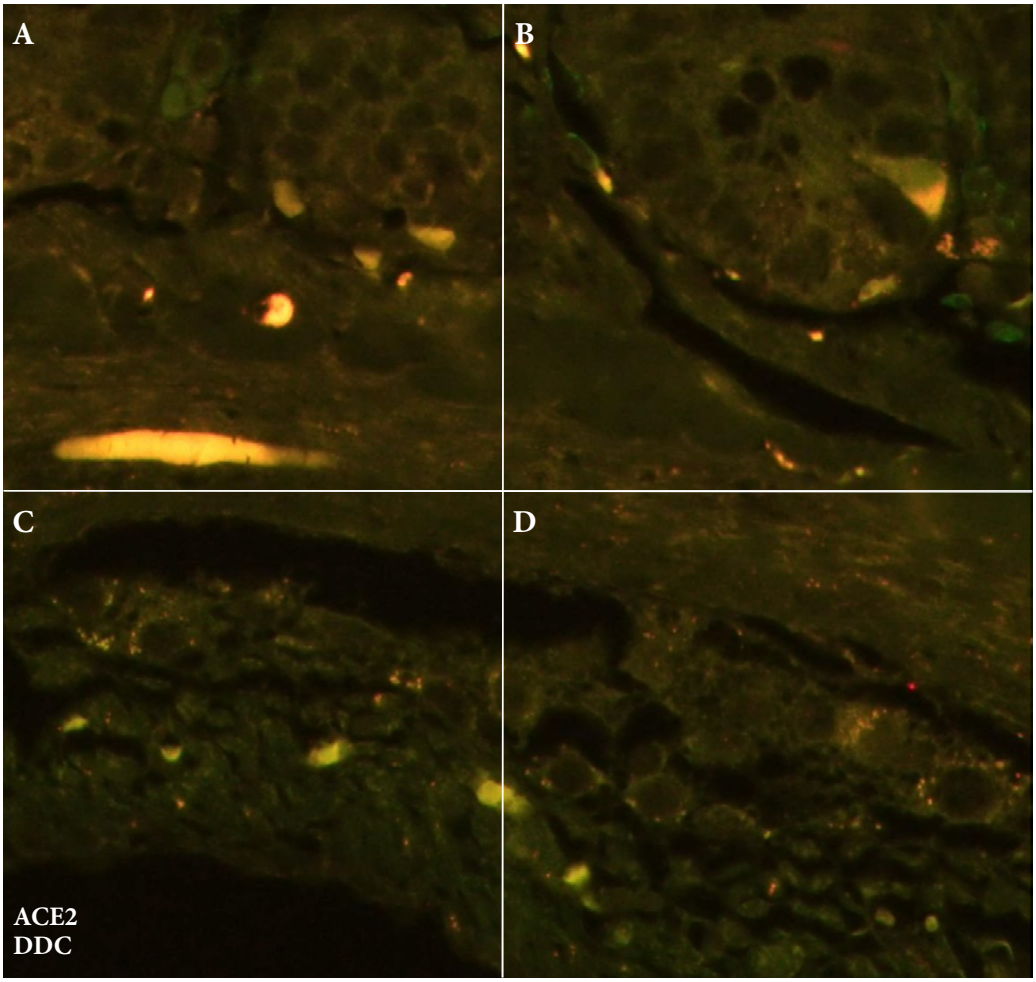

Fig. 4. Representative photomicrographs of ileal cross-sections double-stained for ACE2 and DDC; magnification ×40 (A, B) or ×60 (C,D), Olympus DP74.

(TH, an enzyme responsible for catalyzing the conversion of the amino acid L-tyrosine to L-3,4-dihydroxyphenylalanine), DAT (a sodium-dependent dopamine transporter), VMAT2 (vesicular monoamine transporter 2), and DDC expression indicate dopaminergic phenotype of a neuron. On the other hand, DDC is essential for the formation of dopamine from exogenous L-DOPA; and DDC levels were up-regulated in drug-naive (de novo) patients with Parkinsonian symptoms and in non-treated individuals with preclinical Dementia with Lewy bodies. It was then hypothesized that increased production of DDC in neurons that normally receive dopaminergic input (such as those in the striatum) should be a compensation for low dopaminergic levels [22]. Previously, it was also reported in a rat model that DDC activity was up-regulated soon after lesions in dopaminergic neurons had occurred [23]. Thus, increased levels of DDC could be a marker of reduced dopamine signaling in the brain [22]. Yet, no such data exist with regard to the enteric

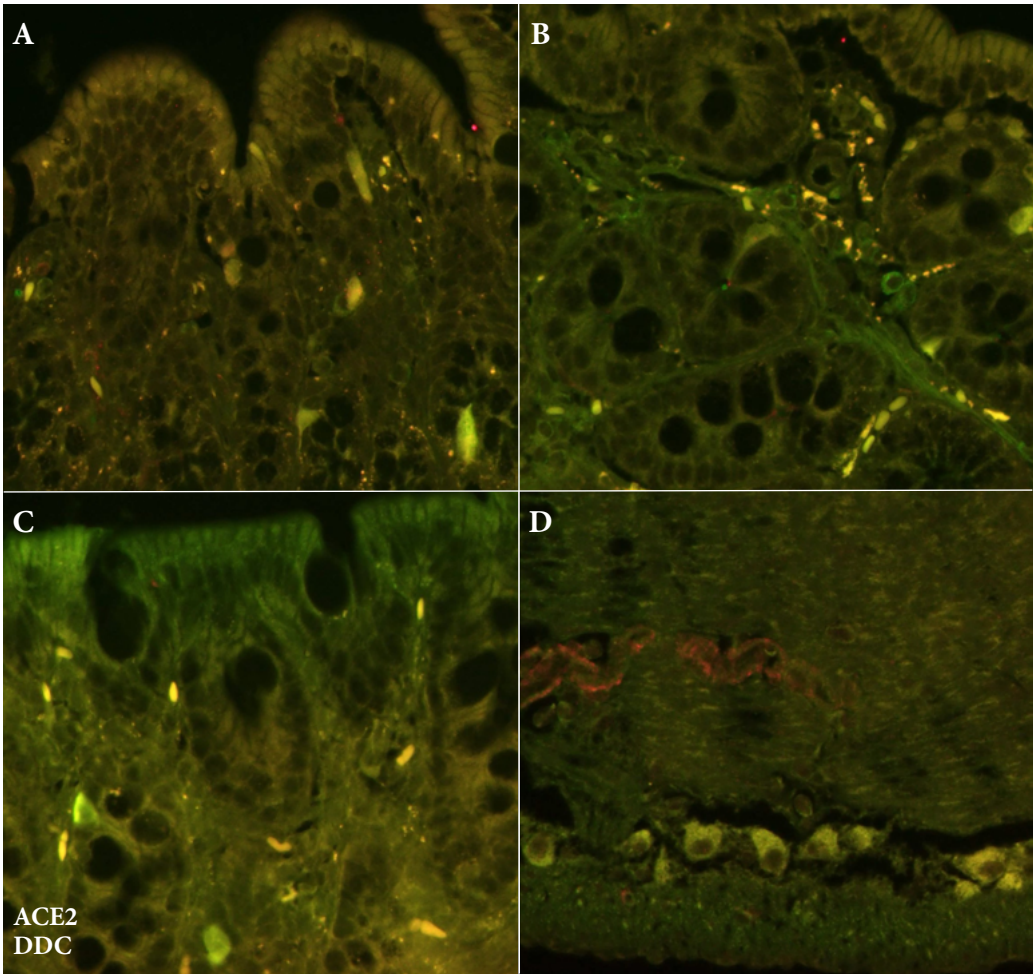

Fig. 5. Representative photomicrographs of colonic cross-sections double-stained for ACE2 and DDC; magnification ×40 (A, B) or ×60 (C,D), Olympus DP74.

nervous system, however, it is estimated that about 50% of the body dopamine is produced in the GI tract by intestinal epithelial cells and enteric neurons [24], and dopamine not only regulates gastroduodenal mucosal barrier but may shape immune regulation in other organs and tissues, too [25]. What is more, ACE2 may also regulate oxidative stress and inflammatory responses in dopaminergic neurons via the mitochondrial ACE2/MrgE/NO axis, which could further influence on intestinal inflammation and neurodegeneration [26].

Conclusions

Co-localization of DDC and ACE2 may imply a direct functional relationship between the GI renin–angiotensin as well as dopaminergic and serotoninergic systems in the regulation of intestinal

permeability, motility and modulation of the gut-brain-axis, which underlines the need for further GI investigation. Both, transgenic animal models and GI tissue sections from patients with gastrointestinal and neurological disorders could provide highly valuable insights into the interaction between renin–angiotensin and monoaminergic systems in the GI tract.

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Disclosures

All authors declare no competing interests.

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