J. Comp. Path. 2014, Vol. 150, 287–296

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EXPERIMENTALLY INDUCED DISEASE

Effects of Inflammation and Axotomy on Expression of Acetylcholine Transferase and Nitric Oxide Synthetase within the Cocaine- and Amphetamine-regulated Transcript-immunoreactive Neurons of the Porcine Descending Colon

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Summary

This study reports changes in expression of acetylcholine transferase (AChT) and nitric oxide synthetase (NOS) in neurons immunoreactive for cocaine- and amphetamine-regulated transcript (CART) peptides during chemically-driven inflammation and axotomy in the porcine descending colon. The co-localization of the neurotransmitters with CART was studied by double immunofluorescence in the myenteric plexus (MP) and outer submucosal plexus (OSP) of the porcine descending colon under physiological and selected pathological conditions. In control animals, neurons expressing CART also expressed AChT in 25.37 ± 0.98% and 26.73 ± 0.96% in the MP and OSP, respectively. Neuronal co-expression of CART with NOS occurred in 90.66 ± 2.13% and 88.09 ± 2.96% in the MP and OSP, respectively. Following axotomy the number of neurons co-expressing CART and AChT decreased to 16.50 ± 3.20% in the MP and increased to 35.49 ± 2.04% in the OSP, while the number of neurons co-expressing CART and NOS increased to 96.66 ± 2.38% in the MP and 97.46 ± 2.22% in the OSP. Experimentally-induced colitis resulted in an increase in the number of neurons co-expressing CART and AChT to 42.40 ± 2.28% in the MP and 63.62 ± 1.83% in the OSP. Similarly, in these animals the number of neurons co-expressing CART and NOS increased to 93.9 ± 2.58% in the MP and 90.43 ± 2.09% in the OSP. Sham-operated controls showed expression levels of 26.22 ± 0.66% (MP) and 27.02 ± 1.73% (OSP) for simultaneous CART and AChT expression and 94.18 ± 0.93% (MP) and 88.21 ± 0.81% (OSP) for CART and NOS co-localization. These data confirm that the examined neurotransmitters have a role in traumatic and inflammatory responses of enteric neurons.

Keywords: acetylcholine transferase; CART; enteric nervous system; nitric oxide synthetase

Introduction

Cocaine- and amphetamine-regulated transcript (CART) peptides, first identified in the ovine hypothalamus (Spiess *et al.*, 1981), are also found within the enteric nervous system (ENS). Expression of CART was confirmed in different parts of the gastrointestinal (GI) tract of numerous species (Ekblad, 2006) including pigs (Gonkowski *et al.*, 2009a) and man (Gunnarsdóttir *et al.*, 2007; Gonkowski *et al.*, 2009b). CART peptides play a role in stress responses (Koylu *et al.*, 2006), feeding behaviour (Kristensen *et al.*, 1998; Hunter *et al.*, 2004), reduction of gastric acid secretion (Okumura *et al.*, 2000) and exacerbation of colonic motility (Tebbe *et al.*, 2004); however, detailed functions of CART

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0021-9975/$ - see front matter
http://dx.doi.org/10.1016/j.jcpa.2013.08.007

within the GI tract, especially under pathological conditions, have not yet been completely elucidated (Gunnarsdóttir *et al.*, 2007; Gonkowski *et al.*, 2009b). Investigation of the co-expression of CART with other biologically active substances may reveal further possible physiological functions of the peptide within the ENS.

Both acetylcholine (ACh) and nitric oxide (NO) have been identified in the descending colon. ACh, the first neurotransmitter discovered and isolated from mammalian intestine (Dikshit, 1938), participates in control of GI motility (Costa and Furness, 1982) and intestinal mucosal function (Tapper, 1983). NO is one of the gaseous neurotransmitters (Kasparek *et al.*, 2008) that inhibits transmission in the sympathetic nervous system (Patel *et al.*, 2001). Its expression and functional significance in ENS neurons have been described in species including pigs (Brown and Timmermans, 2004; Matsumoto *et al.*, 2011) and man (Belai and Burnstock, 2000; Schemann and Neunlist, 2004).

The neurons of enteric ganglia can change their chemical phenotype as a result of adaptive responses to different stimuli, including intestinal and extra-intestinal diseases or nerve injuries (Vasina *et al.*, 2006; Gonkowski *et al.*, 2010). Investigation of the neuronal co-expression of CART and other biologically active substances is therefore relevant to understanding regulation of physiological process as well as the pathogenesis of intestinal diseases in man and animals. The colon is particularly vulnerable to diseases including ulcerative colitis, Crohn's disease, cancer and Hirschsprung's disease (Abad *et al.*, 2003; Gunnarsdóttir *et al.*, 2007). The pig is a major mammalian model for studies of human GI physiology and diseases (Verma *et al.*, 2011).

The aim of the present study was to examine neuronal co-expression of nitric oxide synthetase (NOS) and acetylcholine transferase (AChT) with CART in the ENS of the porcine descending colon in normal animals and following experimental axotomy and induction of colitis.

Materials and Methods

The present study was performed with 16 immature female Large White Polish pigs. The pigs were approximately 10 weeks old and 20 ± 1.0 kg in body weight. They were housed under standard laboratory conditions with access to food and water *ad libitum*. All surgical procedures were performed in compliance with the regulations and rules approved by the Local Ethical Committee (decision number 85/2008).

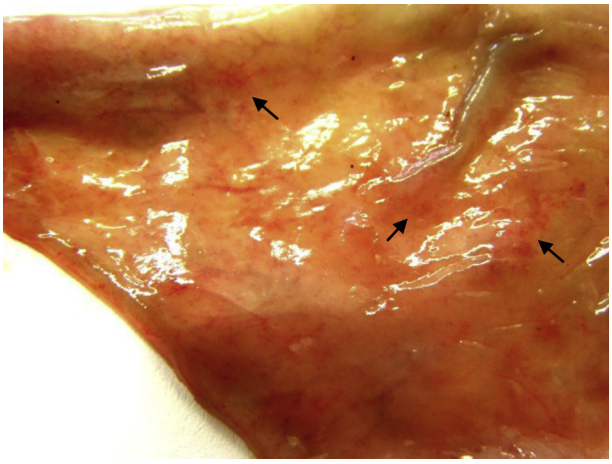

Fig. 1. Descending colon from a pig with induced inflammation. Arrows indicate petechial haemorrhages.

The animals were divided into four groups of four animals each. One control group was composed of untreated animals and the second control group comprised sham-operated pigs that were subjected to laparotomy and an injection of sterile isotonic (0.9%) saline into the wall of the descending colon. The first experimental group underwent induction of aseptic colitis by laparotomy and injection of 10% formalin solution into the wall of the descending colon. The second experimental group had transection of the caudal rectal nerves. Both procedures were performed as described by Burliński (2012). For all surgical procedures animals were premedicated with azaperone (Stresnil, Janssen Pharmaceutical, Beerse, Belgium; 0.2 mg/kg by intramuscular injection). After 20 min the gilts were anaesthetized

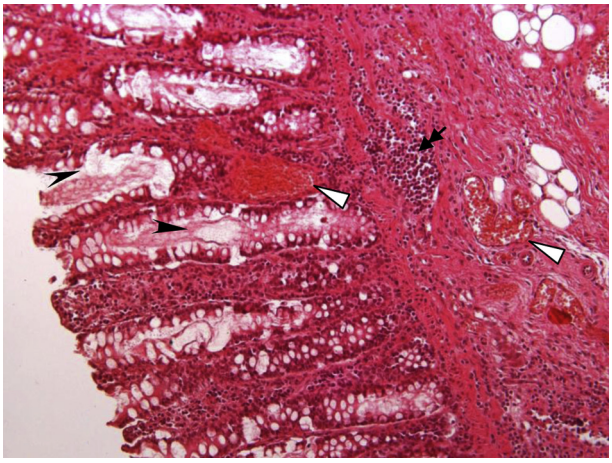

Fig. 2. Descending colon from a pig with induced inflammation. There is a focus of inflammatory cells (double arrow), areas of haemorrhage (white arrowheads) and increased mucus secretion (black arrowheads). HE. ×200.