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Full Length Article

Changes in VIP-, SP- and CGRP- like immunoreactivity in intramural neurons within the pig stomach following supplementation with low and high doses of acrylamide

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ABSTRACT

Acrylamide is one of the food toxins to which the human body is exposed. Although researchers' interest in acrylamide has been growing in recent years, the knowledge of its effect on the gastrointestinal tract, especially on intramural neurons which form the enteric nervous system is scarce. The aim of this experiment was to determine the influence of acrylamide, administered at doses equivalent to the human tolerable daily intake (TDI, 0.5 μg/kg b.w./day) and ten times higher than the TDI (5 μg/kg b.w./day), on the distribution of vasoactive intestinal peptide (VIP), substance P (SP), and calcitonin gene related peptide (CGRP) in intramural neurons of the domestic pig stomach. Using double immunofluorescent labelling we revealed that the ENS neurons underwent adaptive changes in response to the supplementation of acrylamide, which manifested themselves as increased expression of VIP, SP and CGRP, both in intramural neurons and by an increase in the nerve density in submucous and muscular layers in the porcine stomach. These substances take part in defensive reactions of neurons and transmission of sensory reactions may play an important role in protecting the stomach against the harmful effect of acrylamide. Moreover, it has been shown that acrylamide induces a significant response of ENS neurons even in TDI dose, which suggests that it is not neutral to the body. These findings may be the basis for further toxicological studies addressing the question if currently permitted minimal content of acrylamide in the food does jeopardize the health of human consumers?

1. Introduction

The stomach plays a physiologically important role in digestion: it secretes hydrochloric acid, digestive enzymes, mucus and homogenises the chyme. These functions can be performed owing to the involvement of various humoral and nervous pathways (Pimont et al., 2003). Neural control of the gastrointestinal function is exerted by extrinsic innervation - sympathetic, parasympathetic and sensory ganglionic neurons – and intrinsic innervation – intramural neurons which form the enteric nervous system (ENS) (Ekblad and Bauer, 2004; Palus et al., 2017). Neurons located in the gastrointestinal tract wall are grouped in the ganglia interconnected by a dense network of nerves, making up enteric plexuses. ENS regulates motor activity, intestinal excretion, blood flow and acts together with the intestinal endocrine and immune system independently of the central nervous system. Due to its autonomy and the number of neurons which make up the ENS, it is called the "intestinal brain" (Furness et al., 2014). Organisation of ENS is species-specific and depends on the section of the gastrointestinal tract. In the upper section of the gastrointestinal tract (oesophagus and stomach), ENS is divided into two plexuses: the myenteric plexus (MP) located between the circular and the longitudinal muscle layer, and the submucous plexus (SmP), situated near the basal membrane of the mucous membrane (Gonkowski, 2013). The muscular plexus mainly innervates the muscle layer, participating in the regulation of the motor activity of the gastrointestinal tract, but also - to a lesser extent - the neurons serve the mucous membrane and gastrointestinal tract glands. Meanwhile, neurons of the submucosal plexus mainly control the secretory function of the mucosa (Huizinga et al., 2011; Timmermans et al., 2001). The numerous regulatory functions in the gastrointestinal tract can be performed owing to broad spectrum of biological effects of the neuroactive substances synthesised by ENS neurons. Moreover, ENS is very flexible in response to pathological agents and adaptable to changing environmental conditions. Various pathological processes within the gastrointestinal tract, such as inflammation, neuron damage or intoxication can induce a response of ENS neurons expressed by structural changes, changes in excitability or a change of the neurochemical

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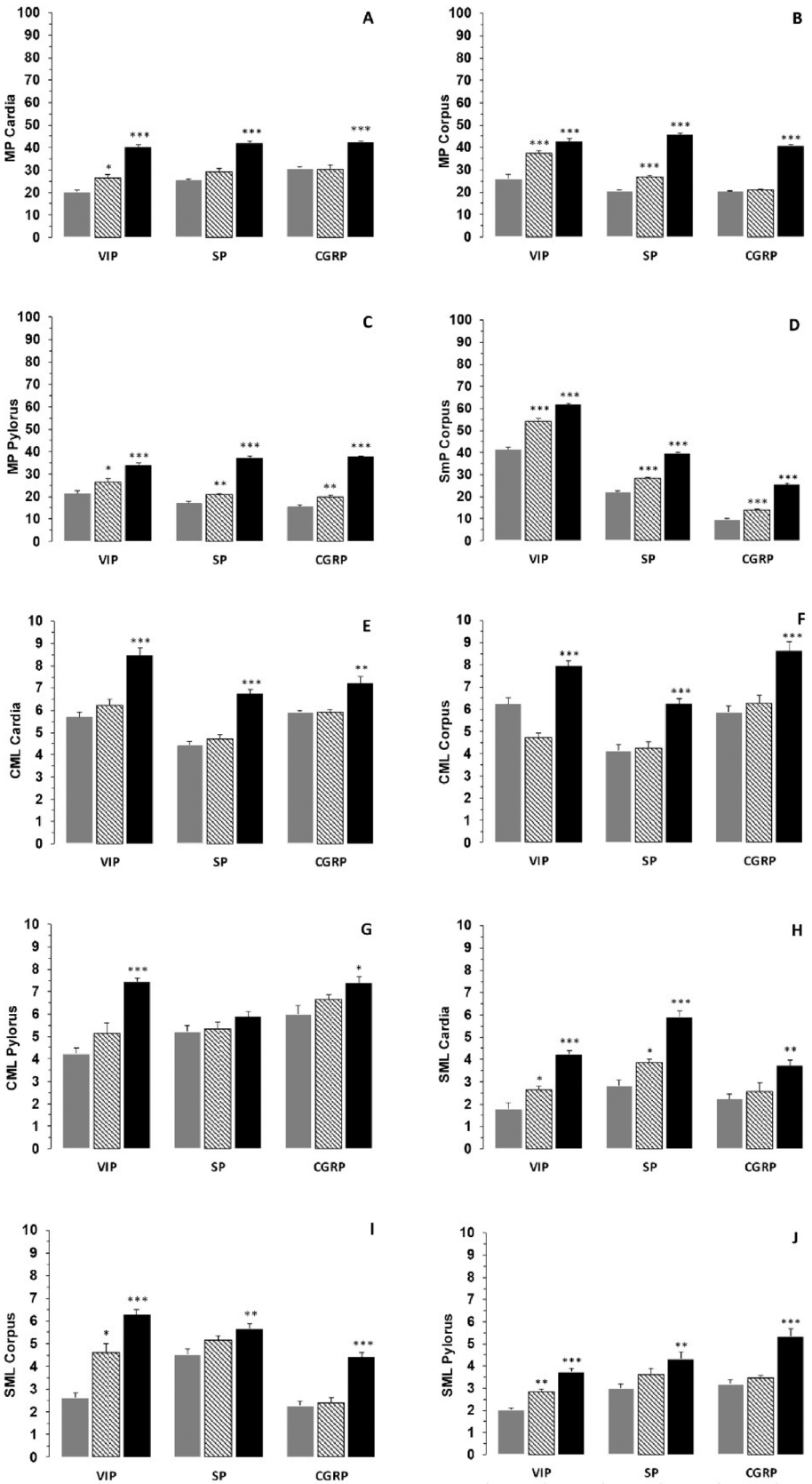

Fig. 1. Acrylamide- induced changes in immunohistochemical profiles of nerve structures in the wall of the porcine stomach.
The ENS neurons in MP displaying immunoreactivity to VIP, SP and CGRP in control (grey bar), LD (lined bar) and HD (black bar) groups: in cardia (A), corpus (B) and pylorus (C) and in SmP in the corpus (D). Nerve fibres in the field of vision within CML displaying immunoreactivity to VIP, SP and CGRP in control (grey bar), LD (lined bar) and HD (black bar) groups: in cardia (E), corpus (F) and pylorus (G). Nerve fibres in the field of vision within SML displaying immunoreactivity to VIP, SP and CGRP in control (grey bar), LD (lined bar) and HD (black bar) groups: in cardia (H), corpus (I) and pylorus (J).
*p < 0.05,**p < 0.01,***p < 0.001 indicate differences in expression of particular substance studied in comparisons to the control animals

phenotype of nerve cells manifesting itself as increasing and/or decreasing expression of some neuroactive substances (Ekblad and Bauer, 2004; Furness et al., 2014; Gonkowski, 2013). Vasoactive intestinal peptide (VIP) is an endogenous, 28-amino-acid neuropeptide, with a broad biological spectrum, commonly occurring both in the central and in the peripheral nervous system, especially in the ENS (Van Ginneken et al., 1996). VIP receptors (VPAC 1 and VPAC2) are members of the family of metabotropic receptors for secretin, glucagon and GHRH and