

The influence of inflammation on the expression of neuropeptides in the ileum-projecting primary sensory neurones in the pig

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[Received 22 May 2003; Accepted 30 June 2003]

In the present study the ELISA test was used to investigate the influence of chemically-induced ileitis on the dorsal root ganglia (DRG) neurons in the pig. The preliminary retrograde fluorescent tracing study revealed that ileum-projecting sensory neurones (IPN) are located in the thoracic ganglia (Th₈–Th₁₃). The ileum wall in experimental (E) pigs was subjected to multiple injection with 4% paraformaldehyde to induce inflammation, while in the control (C) animals the organ was injected with 0.1 M phosphate buffer. Three days later the DRGs (Th₈–Th₁₃) collected from all the animals were evaluated for VIP, SP, CGRP, NPY, GAL and SOM content with an ELISA test. It was found that the inflammation increased clearly the tissue level of SP, GAL and SOM.

key words: DRG, ileum, neuropeptides, chemical Inflammation, pig

INTRODUCTION

The regulation of intestinal functions by the nervous system is increasingly appreciated. Primary afferent neurones respond to noxious mechanical and chemical stimulation and convey information to the central nervous system. However, knowledge of the efferent function of sensory neurones containing sensory neuropeptides is still incomplete. Efferent functions of sensory neuropeptides have been demonstrated in inflammatory reactions in the eye, skin and joints (for details see [4]). The contribution primary afferents make to inflammatory processes in the gastro-intestinal tract is not fully elucidated [5]. There is mounting evidence that sensory neuropeptides are involved in the regulation of inflammatory processes in the gut. It has been revealed that in the pig the sensory nerve fibres in the ileum originate in the thoracic dorsal root ganglia Th₈–Th₁₃. The ileum-projecting neurones (IPN) located in the

above-mentioned ganglia contain immunoreactivity to CGRP, SP, SOM and GAL (Pidsudko, unpublished data).

We therefore decided to study the influence of experimental ileitis on the levels of some neuropeptides of dorsal root ganglia (DRG) neurons in the pig.

MATERIAL AND METHODS

The study was performed on 10 juvenile female pigs (of body weight 10 kg) of the Large White Polish breed. All the animals were housed and treated in accordance with the rules approved by the local Ethical Commission. The surgery was performed as described previously [3]. Chemical inflammation was induced in 5 animals (group E) by injection of 1 ml of 4% paraformaldehyde into the wall of the ileum; the remaining animals (group C, n = 5) were sham-operated and 1 ml of 0.1 M phosphate buffer (pH 7.4) was injected into the wall of ileum. Three days

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Supported by KBN grant 6P06K 012.

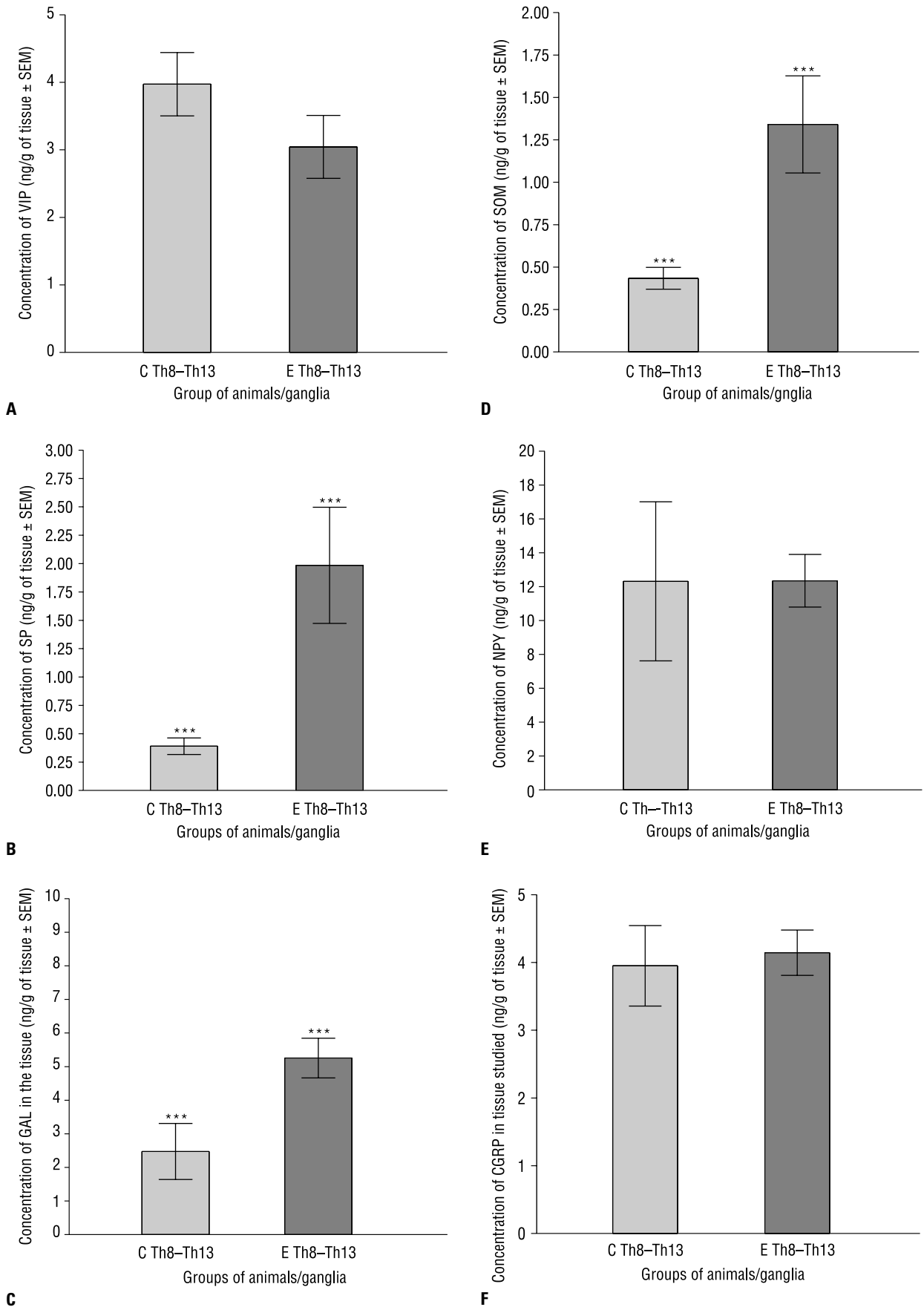


Figure 1. Concentration of the neuropeptides studied in Th₈–Th₁₃ dorsal root ganglia of the control and experimental animals.

Table 1. High sensitivity enzyme immunoassay kits used (Peninsula Laboratories Inc.)

Substance	Code	Lot No.
NPY	S-1220 (EIAH-7172)	015669
VIP	S-1183 (EIAH-7161)	016535
GAL	S-1210 (EIAH-7100)	016537
SOM	S-1179 (EIAH-8001)	016538
SP	S-1180 (EIAH-7451)	016536
β -CGRP	S-1200 (EIAH-6012)	016533

later all the animals were deeply re-anaesthetised and sacrificed by an overdose of pentobarbital. Dorsal root ganglia (Th₈–Th₁₃) were collected, weighed and snap-frozen in liquid nitrogen. Dorsal root ganglia samples were then homogenised with a homogeniser (Ultra Turax). The homogenates were centrifuged for 15 minutes at 10,000 g and supernatant was collected and frozen at -70°C . Tissue concentrations of neuropeptide Y (NPY), vasoactive intestinal polipeptide Laboratories, INC; for details see Table 1). Absorbancy was measured with polypeptide (VIP), galanin (GAL), somatostatin (SOM), substance P (SP) and calcitonin gene-related peptide (CGRP) were determined by ELISA tests (Peninsula immuno-plate reader (polypeptide; Dynex MRX; Dynex Technologies) with a 450 nm filter. A ten-point standard curve was prepared and results were re-calculated per 1 g of wet tissue. The data obtained were statistically analysed with the Student t-test. The differences were considered statistically significant at $p < 0.05$. Data are expressed as means \pm SEM.

RESULTS AND DISCUSSION

Levels of the biologically active substances studied in DRG from normal and inflamed animals are shown in Figure 1. The level of VIP in DRGs of inflamed pigs (2.91 ± 0.47 ng/g) was lower than that found in the control animals (4.07 ± 0.47 ng/g). On the other hand, the level of SP was significantly higher ($P < 0.05$) in the inflamed animals (1.87 ± 0.51 ng/g) than in the control ones (0.36 ± 0.08 ng/g). The level of GAL and SOM was also significantly higher in the inflamed pigs (5.25 ± 0.59 ng/g and 1.16 ± 0.29 ng/g, respectively) than in the control animals (2.46 ± 0.84 ng/g and 0.43 ± 0.06 ng/g, respectively).

Levels of NPY (12.31 ± 4.69 ng/g in controls versus 12.34 ± 1.55 ng/g in experimental animals) and CGRP (3.95 ± 0.6 ng/g in control versus 4.14 ± 0.33 ng/g in experimental animals) in DRGs were not affected by the inflammatory process. These results revealed that ileitis increased levels of SP, SOM and GAL in DRG neurons studied but decreased levels of VIP. Levels of NPY and CGRP did not change.

The present data show that gastro-intestinal disorders involving mucosal infection, inflammation or ulceration can be associated with changes in the peptidergic innervation of the gut. The alterations of neuropeptide expression are variable, and in many cases it is not known whether they are primary or secondary to the disease. They may reflect changes in the expression or metabolism of neuropeptides, changes in nerve activity or changes in peptide release [2]. On the other hand, the biological significance of the afferent extrinsic neuropeptide release is still poorly understood. It was discovered that SP enhances the inflammatory response by stimulating immune cells located in the intestine and by interacting with mast cells. SP may also modulate the inflammatory response via a variety of different cytokines and growth factors (for references see [4]). As an ablation of capsaicin-sensitive sensory neurons [1] causes a significant increase in the severity of experimental colitis in the acute model of the rabbit and the chronic TNB-colitis of the rat [4], it is possible that the sensory nervous system plays a protective role in gut inflammation.

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