Immunohistochemical Study of Gastrointestinal Endocrine Cells in the Porcupine (Hystrix cristata)

M. YAMAN1*, B. GENÇER TARAKÇI1, A. BAYRAKDAR1, O. ATALAR2 and O. DABAK3

1Department of Histology and Embryology, Faculty of Veterinary Medicine, Firat University, 23119 Elazig, TURKEY.
2Department of Anatomy, Faculty of Veterinary Medicine, Firat University, 23119 Elazig, TURKEY.
3Department of Histology and Embryology, Faculty of Medicine, Firat University, 23119 Elazig, TURKEY.

*Corresponding author: E-mail: yaman@firat.edu.tr

SUMMARY

The regional distribution and the relative frequency of endocrine cells was studied immunohistochemically in the gastrointestinal tract of the porcupine (Hystrix cristata), using antiserum against serotonin, calcitonin gene related peptide (CGRP), somatostatin-14, neurotensin, cholecystokinin (CCK) and vasoactive intestinal polypeptide (VIP). Serotonin immunoreactive cells were observed in all regions of the gastrointestinal tract and they were particularly abundant in the stomach and the duodenum, whereas the presence of somatostatin 14 positive cells and of neurotensin positive cells were restricted to intestines (to small and large bowels and only to small intestine, respectively) and CGRP immunoreactive cells were predominantly evidenced in the stomach. By contrast, CCK and VIP immunoreactive cells were not detected in the porcupine.

Keywords: Immunohistochemistry, porcupine, calcitonin gene related peptide, somatostatin-14, neurotensin, cholecystokinin, vasoactive intestinal polypeptide.

RESUME

Etude immunohistochimique des cellules endocrines du tractus gastro-intestinal chez le porc-épic (Hystrix cristata)

La distribution des cellules endocrines ainsi que leur fréquence relative dans le tractus gastro-intestinal du porc-épic (Hystrix cristata) ont été étudiées par immunohistochimie au moyen d’anticorps dirigés contre la sérotonine, le peptide dérivé du gène de la calcitonine (CGRP), la somatostatine 14, la neurotensine, la cholécystokinine (CCK) et le peptide vaso-intestinal (VIP). Des cellules positives pour la sérotonine ont été décelées dans toutes les régions du tractus gastro-intestinal et ont été plus particulièrement abondantes dans l’estomac et le duodénum, alors que les cellules positives pour la somatostatine 14 ou pour la neurotensine ont seulement été observées dans les portions intestinales (somatostatine 14 : intestin grêle et colon ; neurotensine : intestin grêle) et que les cellules positives pour le CGRP ont été essentiellement localisées dans l’estomac. En revanche, aucune cellule immuno-réactive pour la CCK et le VIP n’a été mise en évidence dans le tube digestif du porc-épic.


Introduction

Gastrointestinal endocrine cells that all dispersed in the epithelia and gastric glands of digestive tract synthesize various kinds of gastrointestinal hormones and play important role physiological roles in digestion and nutriment utilisation [4]. The porcupine belongs to the Hystricidae family, which constitutes a small group of the order Rodentia [6, 20]. Although many reports have been carried out on the regional distribution and relative frequency of the different endocrine cells in the gastrointestinal tract of various vertebrates including several species and strains of rodents [19, 21-24, 28, 34], little information concerning hormone production by the porcupine gastrointestinal tract is available and the studies have only investigated serotonin immunoreactivity [37]. Thus, the present study investigated regional distribution and relative frequency of some mediators, commonly produced by the gastrointestinal tract of various vertebrates and various rodent species [19, 21-24, 28] in the endocrine cells of porcupine (Hystrix cristata) gastrointestinal tract by immunohistochemistry.

Material and Methods

ANIMALS AND TISSUE SAMPLES:

Five adult female porcupines (Hystrix cristata) of different ages (2 to 4 years old) and trapped by peasants in Eastern Anatolia (Turkey) were used. Deep anesthesia of animals was induced by initial injection of ketamin HCl (Ketanes 10-15 mg/kg, i.m) followed by xylazine HCl (rompun 0.10-0.15 mg/kg i.m). Tissue samples were taken from stomach, duodenum, ileum and colon by biopsy puncture and fixed in 4% neutral-buffered formalin for 24 hours. Then tissues were dehydrated through graded ethanol and embedded in paraffin. Seven µm thick sections were obtained and processed for immunohistochemical staining.

Revue Méd. Vét., 2007, 158, 4, 196-200
IMMUNOHISTOCHEMISTRY: PAP (PEROXIDASE – ANTI – PEROXIDASE) METHOD:

Immunohistochemical staining was carried out by using the peroxidase-antiperoxidase (PAP) method. Blocking of endogenous peroxidase was carried out with 0.08% hydrogen peroxide (H$_2$O$_2$) in methanol for 5 minutes [36]. In order the block unspecific binding, incubation with 1:10 normal goat serum in 0.1 M phosphate buffered saline (PBS, pH 7.2) was performed.

Sections were incubated for 16-20 hours at 4°C in, rabbit IgG antibodies against serotonin (Zymed Lab., 18.0077), calcitonin gene-related peptide (Chemicon, AB5920), somatostatin-14 (Chemicon, AB1976), vasoactive intestinal polypeptide (Chemicon, AB982), neurotensin (Chemicon, AB5496) and cholecystokinin (Chemicon, AB1973). Antibodies were diluted to 1:20, 1:500, 1:100, 1:500, 1:100 in PBS containing 0.25% sodium azide and 2.5% bovine serum albumin respectively. Sections were then incubated in goat anti-rabbit IgG (Dako, Z0421, Denmark), followed by rabbit peroxidase anti-peroxidase complex (Zymed Lab., 61.2003, San Francisco), both at dilution of 1:50 in PBS, for 1 hour at room temperature. Sections were washed in PBS for 30 minutes after each incubation, and finally immersed in glucose oxidise-Diaminobenzidine (DAB)-nickel ammonium sulphate substrate [30] for 10 minutes. After washing in distilled water and counterstaining with eosin, sections were dehydrated and cover slips mounted with aqueous permanent mounting medium.

The specificity of each immunohistochemical reaction was determined as recommended by STERNBERGER [35] by using (including the replacement of) specific antiserum preincubated with its corresponding antigen. Sections were examined with light microscope and photographs were taken.

Mean numbers of immunopositive cells in each sample obtained from the different organs were determined by counting the positive cells in five randomly selected microscopic fields with 40X magnification (numbers of immunopositive cells / microscopic field). Then the arithmetic means were calculated for each sample.

Results

Serotonin, CGRP, somatostatin-14 and neurotensin immunoreactive cells were found in the gastrointestinal tract of the porcupine (Hystrix cristata) and CCK and VIP immunoreactive cells were not detected in any region of the porcine gastrointestinal tract. Nevertheless, VIP immunoreactivity was only observed in nerve fibers of duodenum and colon (figure 1). Most of these immunoreactive cells were generally round to spherical-shaped close type cells and spherical to spindle-shaped open type cells situated in the stomach regions and intestinal gland regions.

Serotonin immunoreactive cells were encountered in all parts of the gastrointestinal tract, particularly in the stomach (cardia and pylorus) and in the duodenum (Table I). In the other intestinal regions, their relative frequency was low.

Open types of cells were restricted to the interepithelial regions while most of the closed type cells were found in the stomach and intestinal gland regions (Figure 2). Neurotensin positive spindle shaped cells were observed exclusively in the small intestine (duodenum and ileum) (figure 3) with a moderate frequency, whereas somatostatin 14 positive cells, usually spindling shaped, were located throughout the intestines (small and large bowels) with a low frequency (figure 4) and were totally absent in the stomach (Table I). By contrast, CGRP immunoreactive cells were mostly evidenced in the stomach (cardia and fundus) and scarcely in the ileum (Table I). Most of these cells were closed type cells (figure 5).

The distribution pattern of the endocrine cells in the gastrointestinal tract was identical in the 5 tested animals (table I).

Discussion

The distribution of each endocrine type cells (localisation and frequency) in the gastrointestinal tract of the porcupine was remarkably different. Serotonin immunoreactive cells were evidenced throughout the gastrointestinal tract whereas the localisation of the other endocrine cells appeared more tissue specific: CGRP cells were predominant in the stomach, somatostatin 14 was only produced by intestinal cells and neurotensin expression was restricted to the small intestine.

Serotonin is a monoamine and is widely distributed in nervous system, in gastrointestinal tract and in endocrine pancreas [7] where serotonin mainly inhibits gastric acid secretion and contraction of smooth muscle in the gastrointestinal tract [11]. Serotonin immunoreactive cells were detected throughout the gastrointestinal tract of all species and in the gastrointestinal tract at the early stage of vertebrate evolution [7]. In addition, these immunoreactive cells were detected in the whole alimentary tract including oesophagus of low vertebrates [18]. Serotonin immunoreactive cells were detected in the whole gastrointestinal tract of gerbil [24], common tree shrew [38], Philippine carabao [3], Manchurian chipmunk [23], rat [14], mouse [19, 29] and porcupine [37]. In the present study, serotonin immunoreactive cells were detected throughout the whole gastrointestinal tract and showed the highest frequencies in the cardiac region of stomach and duodenum. These results are line with previous studies.

CGRP is a neuropeptide constituted by 37 amino acids that is encoded by alternative splicing products of the calcitonin gene [2]. In the stomach, CGRP is contained in the peripheral endings of capsaicin-sensitive afferent nerves [10]. However, in the present study, CGRP immunoreactivity was detected in endocrine cells of cardiac and fundus region of porcupine stomach with rare frequency. Studies have shown that CGRP has gastro-protective effects through various possible mechanisms [13, 26]. Recently, the localisation of calcitonin gene related peptide receptors in the rat gastric mucosa (probably on D cells) suggested that CGRP may have gastro protective effects by stimulating the production and secretion of somatostatin by D cells via its receptors [15]. This protective effect...
may not depend to somatostatin mediated mechanism in porcupine, because somatostatin positive endocrine cells were not detected in porcupine stomach.

The straight and cyclic forms of somatostatin, consisting of 14 amino acids were isolated from the hypothalamus of the sheep for the first time [5], where somatostatin inhibits the secretion of other neuroendocrine hormones [16]. Somatostatin immunoreactive cells are widely distributed in the gastrointestinal tract, except for the large intestine of all vertebrate species [8]. However, some specie dependant variations on the distribution pattern of these immunoreactive cells have been reported. In the gastrointestinal tract of Manchurian chipmunk, positive cells were detected throughout the entire gastrointestinal tract and showed the highest frequency in the pylorus [23] and they were restricted to pylorus region in the gerbil stomach [24]. In the porcupine, somatostatin immunoreactive cells were detected from duodenum to colon with low frequencies.

Neurotensin was initially isolated from the hypothalamus and subsequently localized to endocrine type cells in the ileal mucosa [32]. Eighty to ninety percent of neurotensin is found in the gut, predominantly in the distal jejunum and ileum [9, 27]. In the porcupine, neurotensin immunoreactive cells were restricted to duodenum and ileum. These distributions well corresponded to previous reports [17, 30, 31].

Generally, CCK immunoreactive cells are located in the gastric mucosa and the entire small intestinal tract in mammals [33, 38], LEE et al. [23] reported that CCK immunoreactive cells were abundant in the pylorus gland region but scarce in the duodenum and no cell was found in the other gastrointestinal regions of the Korean tree squirrel. In the tree shrew [38] CCK-immunoreactive cells were restricted to
small intestine with very low frequencies. In the present study, CCK immunoreactive cells were not detected in the porcupine gastrointestinal tract. These differences might be due to different antisera, methods and/or species used in each study.

VIP-immunoreactive nerve fibers have been widely described in all layers of the gut wall in mammals [1, 12, 25]. In the present study VIP immunoreactivity was only observed in nerve fibers in some fields of porcupine gastrointestinal tract as described in previously studies [1, 12, 25].

The distribution and relative frequency of the 4 types (serotonin, CGRP, somatostatin-14 and neurotensin) of immunoreactive cells observed in the porcupine gastrointestinal tract in this study well correspond to previous reports on rodent species, suggesting that the development of endocrine functions into the digestive system is an old event.

**References**


**TABLE 1:** Distribution and relative frequency of serotonin, neurotensin, somatostatin 14, Calcitonin Gene Related Peptide (CGRP), Cholecystokinin (CCK) and Vasoactive Intestinal Polypeptide (VIP) immunoreactive endocrine cells in the porcupine (*Hystrix cristata*) gastrointestinal tract (n = 5).

Results are expressed as means ± standard deviations.

<table>
<thead>
<tr>
<th>Gastrointestinal Tract regions</th>
<th>Animals Mean</th>
<th>Female 1</th>
<th>Female 2</th>
<th>Female 3</th>
<th>Female 4</th>
<th>Female 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serotonin</td>
<td>34 ± 3</td>
<td>28</td>
<td>32</td>
<td>35</td>
<td>32</td>
<td>32 ± 3</td>
</tr>
<tr>
<td>Neurotensin</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Somatostatin 14</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CGRP</td>
<td>10 ± 2</td>
<td>12</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>12 ± 3</td>
</tr>
<tr>
<td>CCK</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>VIP</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

| Small intestine: Duodenum     |              |         |         |         |         |         |
| Serotonin                     | 30 ± 3       | 32      | 35      | 28      | 35      | 32 ± 3  |
| Neurotensin                   | 12 ± 2       | 15      | 14      | 10      | 12      | 13 ± 2  |
| Somatostatin 14               | 5 ± 1        | 6       | 4       | 6       | 5       | 5 ± 1   |
| CGRP                          | 0            | 0       | 0       | 0       | 0       | 0       |
| CCK                           | 0            | 0       | 0       | 0       | 0       | 0       |
| VIP                           | 0            | 0       | 0       | 0       | 0       | 0       |

| Small intestine: Ileum        |              |         |         |         |         |         |
| Serotonin                     | 2 ± 1        | 4       | 7       | 4       | 2       | 4 ± 2   |
| Neurotensin                   | 10 ± 2       | 13      | 11      | 14      | 10      | 12 ± 2  |
| Somatostatin 14               | 5 ± 1        | 8       | 6       | 5       | 6       | 6 ± 1   |
| CGRP                          | 1 ± 1        | 2       | 0       | 1       | 1       | 1 ± 1   |
| CCK                           | 0            | 0       | 0       | 0       | 0       | 0       |
| VIP                           | 0            | 0       | 0       | 0       | 0       | 0       |

| Large intestine               |              |         |         |         |         |         |
| Serotonin                     | 3 ± 1        | 3       | 2       | 1       | 4       | 3 ± 1   |
| Neurotensin                   | 0            | 0       | 0       | 0       | 0       | 0       |
| Somatostatin 14               | 4 ± 2        | 7       | 8       | 5       | 5       | 6 ± 2   |
| CGRP                          | 0            | 0       | 0       | 0       | 0       | 0       |
| CCK                           | 0            | 0       | 0       | 0       | 0       | 0       |
| VIP                           | 0            | 0       | 0       | 0       | 0       | 0       |
200 YAMAN (M.) AND COLLABORATORS
