Immunohistochemical Localization on 5-HT Immunoreactive Cells in Digestive Tract of Dinodon rufazonatum

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Abstract: The morphological features and relative frequency of 5-hydroxytryptamine (5-HT) cells in the digestive tract of the Dinodon rufazonatum were investigated by avidin-biotin-peroxidase complex immunohistochemical methods using 5-HT antisera. Mucosal endocrine cells reacting specifically with the antisera to 5-HT were detectable throughout the digestive tract, with the highest density in the duodenum, secondly in the stomachus pyloricus, and lowest in the oesophagus and stomach corpus. Round or oval cells named closed-type cells were found throughout the whole digestive tract, and mainly located in the oesophagus, stomach and rectum. Open-type cells those had cell processes were mostly present in the intestine except for rectum with a spindle-like, triangular or irregular shape, and occasionally observed in the other regions. We concluded the general distribution pattern of 5-HT cells in the digestive tract of snakes, and discussed the distributive pattern of 5-HT cells in Dinodon rufazonatum combined with it’s life environment and the feeding habit.

Key words: Dinodon rufazonatum; Digestive tract; 5-HT cell; Immunohistochemistry

5-HT is a kind of peptoid, its molecular structure is relatively simple, belongs to single chain. In vertebrate animals, 5-HT immunoreactive cells are widely distributed in the digestive tract and other organs, and have been found in various species. Studies have shown that 5-HT plays an important role in the physiological activity of the digestive tract. In this study, we investigated the 5-HT immunoreactive cells in the digestive tract of Dinodon rufazonatum using the avidin-biotin-peroxidase complex immunohistochemical method. We found that 5-HT immunoreactive cells were present throughout the digestive tract, with the highest density in the duodenum and the stomach, the lowest in the oesophagus and stomach corpus. The distribution pattern of 5-HT cells was different from that in other species, and the results were discussed in the context of the life environment and feeding habit of Dinodon rufazonatum.
1.2 主要试剂

即用型（ready to use）5-HT 抗血清（兔抗人）和链霉素抗生物素卵白-过氧化物酶（streptavidin peroxidase complex，SP）免疫组织化学试剂盒购于北京中山生物技术有限公司，由美国 ZYMED 公司生产，产品代号为 ZA-0231。

1.3 免疫组织化学方法

以 ABC（avidin-biotin-eroxidase complex）免疫组织化学试剂盒（ZYMED 公司，1986）对 5-HT 细胞进行定位。一抗（激素抗血清）孵育条件为 4℃，16 h，生物素化二抗（均为羊抗兔 IgG 抗血清）和 ABC 的孵育均为室温 1 h。DAB-H2O2 显色后，苏木精染色，常规脱水透明，封片。对照实验采用正常血清和 PBS 代替一抗，其余步骤同上。[5]。在 Olympus BH-2 型显微镜下观察，拍摄，并对每个部位随机取 50 个 400 倍视野进行计数（每间隔 10 张切片计数 1 张切片上的阳性细胞数，共计计数 5 张，每张计数 10 个视野），取平均值（M ± SE）表示内分泌细胞的分布密度。

2 结果

表 1 赤链蛇消化道 5-HT 免疫活性细胞的分布密度

<table>
<thead>
<tr>
<th>组织</th>
<th>Duodenum</th>
<th>Jejunum</th>
<th>Ileum</th>
<th>Rectum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osophagus</td>
<td>4.4 ± 0.3^d</td>
<td>7.5 ± 0.4^e</td>
<td>6.4 ± 0.4^c</td>
<td>14.3 ± 0.4^b</td>
</tr>
<tr>
<td>Stomach</td>
<td>19.5 ± 0.7^a</td>
<td>7.8 ± 0.5^e</td>
<td>8.8 ± 0.4^c</td>
<td>8.3 ± 0.3^e</td>
</tr>
</tbody>
</table>

注：经 Duncan’s 检验，上标不同表示平均值之间差异显著（P < 0.05）。By Duncan’s test, different superscripts represented significant differences (P < 0.05).

3 讨论


赤链蛇消化道5-HT细胞的形态学特征，与多数爬行动物的结果一致，即食道、胃部和直肠5-HT细胞大多为闭合型细胞，少数为开放型细胞，肠段5-HT细胞多数属于开放型细胞，少数是闭合型细胞。这些结果暗示5-HT细胞可能行使多种生理功能，在胃部主要起内分泌和旁分泌作用，在肠道各段主要起旁分泌和外分泌作用。遗忘消化道5-HT细胞在形态学上的适应性变化，我们推测，这种变化可能提示着5-HT在胃部和肠道所起的作用不同。

综上所述，赤链蛇消化道5-HT细胞的分布型表现出了很好的与其消化道结构和食性相适应的特点，不但具有爬行动物的共性，而且具有自身独特的分布特点。迄今为止，尚未发现消化道内分泌细胞分布型完全相同的两种爬行动物。我们认为，不同的生活环境对蛇类消化道5-HT细胞的分布型有一定的影响，但食物组成和消化生理状态不同更可能是影响5-HT细胞分布型和形态学特征变化的主要因素，关于具体机制仍有待于进一步研究确认。

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张志强，吴孝兵：赤链蛇消化道5-羟色胺细胞的免疫组织化学定位

5-HT细胞在赤链蛇消化道分布特点和形态学特点（说明详见正文）