神经肽 Y 和 β-内啡肽内分泌细胞在鲻鱼肠道中的分布和形态

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摘要: 用链霉亲合素-生物素-过氧化物酶复合物(Strept Avidin-Biotin peroxidase Complex, SABC)免疫细胞化学方法和兔抗人神经肽 Y 与兔抗人β-内啡肽抗体对鲻鱼肠道不同部位的内分泌细胞进行鉴别和定位研究。结果显示, 这两种神经肽的免疫活性内分泌细胞不同程度地分布在鲻鱼前肠前段和后段、中肠和后肠。神经肽 Y 免疫活性细胞的形态多样, 大多数属开放型细胞, 具有胞质突起, 少数为封闭型细胞, 阳性细胞一般出现在肠褶的中部和近端部。β-内啡肽免疫活性细胞则几乎为封闭型细胞, 且定位在肠褶基部。神经肽 Y 主要分布在前肠前段和后段, 分布密度分别为18.7个细胞/mm² 和 26.3 个细胞/mm², 而在中肠和后肠仅少量分布(< 5.5 个细胞/mm²)。β-内啡肽的分布密度在后肠最高, 达 31.5 个细胞/mm², 其次从前肠前段至中肠顺序递减。还讨论了这两种神经肽在鲻鱼肠道中的生理作用。

关键词: 鲻鱼; 神经肽 Y; β-内啡肽; 免疫细胞化学
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神经肽 Y(Neuropeptide Y, NPY)是胰多肽家族的一个成员, 由 Tatemoto 等首先从猪脑中分离出来, 具有 36 个氨基酸的酰胺肽[1]。β-内啡肽(β-endorphin, β-Ep)为阿片样肽家族一个成员, 是从脑垂体分离出来的β-脂蛋白(β-lipoprotein, β-LPH)羧基末端系列的一部分(61—91 位氨基酸), 其生物合成前身物是β-脂蛋白[2]。生物化学和免疫组织化学技术研究揭示, 神经肽 Y 广泛分布在脊椎动物(从鱼类至哺乳类)的神经系统(脑与脊髓)、脑垂体和视网膜等[3,4]部位, β-内啡肽在脊椎动物的分布与神经肽 Y 类似[5,6]。这两种肽被视为神经递质(Neurotransmitter)、神经调节者(Neuromodulator)或神经激素(Neurohormone)。它们具有多种生理作用, 如控制脑垂体激素的释放、控制心率、记忆、食物和水分的摄取、调节摄食与能量平衡以及血浆中胰岛素的水平等。这两种肽在胃肠中的分布, 仅见 Chiba[7,8] 报道了神经肽 Y 与 5-羟色胺(5-HT)共存于虎纹猫鲨(*Scyliorhinus torazame*)胃肠上皮中, 和 Zhang[9] 报道了 β-内啡肽在兔的胰腺中。而这两种肽在硬骨鱼类胃肠中的分布, 查阅到可利用资料很少。本文用免疫细胞化学方法对神经肽 Y 和 β-内啡肽在鲻鱼肠道各部分进行鉴别和定位, 结果发现在鲻鱼肠道中均有分布, 现报道如下。

1 材料与方法

1.1 材料 5 尾鲻鱼(*Mugil cephalus* Linnaeus)取自福建省龙海市大径海水养殖场, 体长21.5—24.7cm, 体重 190—225g。先在实验室水族箱中暂养 2d, 待消化道排空后活体解剖, 小心分离出肠道, 按前肠前段、前肠后段、中肠、后肠四个部位取材, 迅速投入新配制不含醋酸 Bouin 液中固定 8—12h, 系列酒精脱水, 石蜡包埋, 连续作横切片, 厚 6μm。

1.2 免疫细胞化学反应 实验切片脱蜡去水, 入 3% H_2O_2 中 20min 以除去内源性过氧化物酶活性, 接着按免疫细胞化学 SABC 法(Strept Avidin-Biotin Complex)进行反应。正常羊血清(1∶10)封闭后, 滴加一抗, 分别是兔抗人神经肽 Y 抗体(Sigma 公司产品, 1∶1000 稀释)和兔抗人β-内啡肽抗体(Sigma 公司产品, 1∶400 稀释), 4℃孵育 24h, PBS 洗三次, 加生物素化标记羊抗兔抗体(1∶100)孵育 20min, PBS 洗三次, 加 ABC 复合物(1∶100), 室温下孵育 30min, 最后用 3′, 3′二氨基联苯胺(DAB)显色 10—30min。

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对照试验: 用相邻切片以 PBS 或正常兔血清替代一抗孵育, 结果为阴性。

1.3 观察和计数 切片免疫反应染色后, 在光学显微镜下观察并计数整个横切面中活性细胞的数量, 用目微尺测量并计算横切面的面积。肠道各部位的免疫活性细胞的分布密度(个细胞/mm^2), 以计数 5 尾鱼取其平均值。

2 结果

2.1 鲻鱼肠道中 **NPY** 免疫阳性细胞的形态和分布特点

SABC 免疫染色结果显示, NPY 免疫活性细胞呈深棕色或棕黑色, 轮廓清晰, 背底不着色, 反差大, 极易识别。NPY 细胞散在地穿插于肠黏膜上皮细胞和杯状细胞之间。免疫阳性物质定位在胞质, 胞核不着色。在前肠前段, NPY 免疫活性细胞多数位于肠褶的中部和近端部。细胞形态多样, 多数具有胞质突起, 为开放型细胞。有的为长梭形, 胞体略膨大, 有细长的胞突伸向肠腔(图版Ⅰ: 1, 2); 有的呈柱形, 顶端胞突伸向肠腔(图版Ⅰ: 3); 还有呈烧瓶形, 基部膨大近似锥形, 胞质突起向肠腔(图版Ⅰ: 1, 3); 还可见葫芦形细胞(图版Ⅰ: 3)和较为粗短的锥形细胞(图版Ⅰ: 3, 4)。细胞胞质突起的形态也是多样, 有的伸向肠腔的顶端胞突较长, 延伸至肠腔面(图版Ⅰ: 2); 有的胞突顶部略膨大(图版Ⅰ: 3); 细胞基部胞突较粗短, 呈突触状与基膜接触(图版Ⅰ: 1, 3)。此外, 在肠褶基部, 可见卵圆形或椭圆形的 NPY 免疫活性细胞, 没有胞质突起, 为封闭型细胞(图版Ⅰ: 5)。在前肠后段, 可见形态多样的开放型细胞, 有柱形、烧瓶形、葫芦形(图版Ⅰ: 6, 7)和长梭形(图版Ⅰ: 8), 胞体多位于上皮基部, 顶端突起伸向肠腔, 或与邻近的上皮细胞接触(图版Ⅰ: 7)。个别封闭型细胞也可见。中肠的 NPY 免疫活性细胞均为柱形或长柱形的开放型细胞, 顶端胞突伸向肠腔或邻近的上皮细胞(图版Ⅰ: 9, 10)。后肠中 NPY 免疫阳性反应明显减弱, 免疫活性细胞染浅褐色。

2.2 鲻鱼肠道中 **β-Ep** 免疫阳性细胞的形态和分布特点

免疫细胞化学反应显示, β-Ep 免疫活性内分泌细胞染棕褐色。与 NPY 免疫活性细胞不同, β-Ep 免疫活性细胞主要分布在肠褶基部, 少量稀疏分布在肠褶中部; 细胞为卵圆形或椭圆形, 不具胞质突起(图版Ⅰ: 11—15)。

2.3 **NPY** 和 **β-Ep** 免疫阳性细胞在鲻鱼肠道各部的分布密度

NPY 免疫活性细胞的分布密度在鲻鱼肠道各部位显示不均匀的分布(表 1), 主要分布在前肠, 且后段的密度高于前段, 分别为 26.3 和 18.7 个细胞/mm^2。在中肠, NPY 免疫活性细胞的密度显著减少至 5.1 个细胞/mm^2, 后肠仅见零星呈弱免疫阳性的细胞(4.2 个细胞/mm^2)。

β-Ep 内分泌细胞在肠段的分布密度呈两头高中间低的特点(表 1)。后肠的分布密度最高, 其次从前肠前段至中肠顺序递减。前肠前段和后肠的分布密度分别为 26.9 和 31.5 个细胞/mm^2, 两者没有显著差异。前肠后段和中肠的内分泌细胞数量显著少于前两段, 分布密度为 11.6 和 9.1 个细胞/mm^2。

表 **1** **NPY** 和 **β-Ep** 免疫活性细胞在鲻鱼肠道各部的分布密度(个/mm^2)

Tab.1 Localization density (cells/mm^2) of NPY-ir and β-Ep-ir cells in various parts of intestinal tract in grey mullet

种类 Species	前肠前段 Anterior segment of anterior intestine	前肠后段 Posterior segment of anterior intestine	中肠 Midgut	后肠 Posterior intestine
NPY	18.7±4.3	26.3±5.4	5.1±0.8	4.2±1.0
β-Ep	26.9±7.5	11.6±7.0	9.1±2.7	31.5±11.1

3 讨论

研究结果表明, 神经肽 Y 样和 β-内啡肽样免疫活性内分泌细胞普遍分布在鲻鱼肠道各部分, 即前肠前段、前肠后段、中肠和后肠。这两种内分泌细胞的形态和分布特点与国内外学者对硬骨鱼类(淡水和海水鱼类)胃肠道内分泌细胞的研究报道[10,11]十分类似。它们的组织学特点与潘黔生等[12]的描述相一致, 都是以单个细胞的形式, 散在地分布于肠黏膜上皮细胞和杯状细胞之间。

国外许多学者[1—6]认为, 神经肽 Y 和β-内啡肽的生理作用, 纯属通过中枢性调节机制, 直接或间接影响脊椎动物(从鱼类至哺乳类)的种种生理和代谢活动, 如心率、血液循环、摄食与能量平衡以及脑垂体各种促激素的分泌与抑制活动。至于这两种神经肽在硬骨鱼类肠道中的分布及其生理作用, 几乎查阅不到可利用资料。Gómez-Visus 等[13]报道, 神经肽 Y 与胰高血糖素共同存在于一种鱼 *Dicentrarchus labrax* 的胃和前肠中。Chiba[7]报道, 神经肽 Y 与 5-HT 共存于孵化后不久的虎纹猫鲨的胃肠上皮中。而β-内啡肽在鱼类胃肠道中的分布则未见报道。根据神经肽 Y 在鳙鱼肠道中分布特点和细胞形态类型, 尤其在前肠的分布密度高, 中肠和后肠稀疏分布, 并伸出长的胞质突起通向肠腔, 提示这种神经肽可能的作用是: 参与调节肠道的蠕动和消化吸收以及摄食的调控。β-内啡肽内分泌细胞的形态几乎都是封闭型, 即缺乏胞突。难以理解的是, 为什么不存在开放型细胞, 如果按方之平等认为封闭型细胞很可能是切片所致[10], 那么也不应该全部都是一种形态和几乎是类似分布部位。至于这种内分泌细胞可能的生理作用, 推测是局部参与调节肠道中其他内分泌细胞与腺细胞的分泌与抑制活动, 协调肠道的功能与微环境的稳定。另外, 这两种神经肽在肠道的分布可为证明这些肽的进化保守性提供依据[14]。当然, 关于这两种神经肽确切的生理作用还须进一进深入研究, 以获得更多的实验证据。本文仅提供这两种肽在鳙鱼肠道中分布的形态学证据。

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DISTRIBUTION AND MORPHOLOGY OF NEUROPEPTIDE Y AND β-ENDORPHIN ENDOCRINE CELLS IN THE GUT OF GREY MULLET, *MUGIL CEPHALUS* L.

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Abstract: Neuropeptide Y (NPY), an amidated 36-amino-acid peptide and a member of the pancreatic polypeptide family, was first isolated from porcine brain. β-endorphin(β-EP), a member of opioid peptide family, was isolated from the pituitary gland as a fragment of the carboxyl-terminal sequence of β-eipotropin consisting of 61-91 amino acids. Biochemical and immunochemical studies have shown that NPY and β-EP are widely distributed in the nervous system (brain and spinal cord), pituitary and retina of vertebrates from fishes to mammals, and involved in a wide array of physiological effects, such as regulation of the secretion of piturtary hormones, control of circadian rhythms, memory processing, food and water intake, regulation of feeding, energy balance and plasma insulin levels. Available information on their distribution in the gastroenteritic tract is much restricted. Chiba (1998) reported the coexistence of sertonin and neuropeptide Y in the gut epithelium of the cloudy dogfish, *Scyliorhinus torazame*. Zhang et al. (1994) investigated the distribution of β-EP in the pancreas of rabbit. As to the teleost, little information was available. In the present study, the identification and localization of endocrine cells in the various parts of intestinal tract in grey mullet, *Mugil cephalus* were studied by immunocytochemical method of Strept Avidin-Biotin-Complex (SABC) and two kinds of rabbit antisera raised against mammalian neuropeptide Y and β-endorphin.

The grey mullet *Mugil cephalus*, used in the present study were collected from Dajing Mariculture Farm in Ronghai, Fujian Province. The fish were 21.5—24.7 cm and weighted 90—225g. They were kept deprived of food in aquarium for 2 days. The fish were vivisected, guts were detached and divided into four blocks induding anterior part of anterior intestine, posterior part of anterior intestine, midgut and posterior intestine. The tissue blocks were fixed in Bouin's solution without acetic acid for 8—12h, then dehydrated in a series of alcohol and embeded in paraffin.

Sections at a thickness of 6 um in transverse plane were immunocytochemically stained with a commercial kit using the Strept Avidin-Biotin-Complex (SABC) method. The sections were de-waxed, hydrated and incubated in 3% H_2O_2 for 20 min to remove endogenous peroxidase activity. Incubation was performed in the primary antibodies, rabbit anti-NPY antibody (1∶1000) and rabbit anti-β-EP antibody (1∶400), at 4℃ for 24 h. After three washes in PBS, sections were incubated in the secondary antibody (biotinylated goat anti-rabbit immunoglobulin G, 1∶100) for 30 min at room temperature, then rinsed in PBS three times and incubated for 30 min at room temperature with strep avidin-biotin-peroxidase complex(ABC) The tissue-bound peroxidase was visualized for 10—30 min with diaminobenzidine (DAB) method.

Immunocytochemistry showed NPY and β-EP immunoreactive cells were localized at varying degrees in the anterior part and posterior part of anterior intestine, midgut and posterior intestine. Immunoreactive cells brown, on a unstained background, were easily identified. NPY-immunoreactive cells, majority of which were open-type with cytoplasmic protrusion and minority of which were close-type, had polymorphi and were mainly localized in the middle and apical part of intestinal fold and minorly in the basic part of intestinal fold. Conversly, β-endorphin-immunoreactive cells were almost close-type and were mainly localized in the basic part of intestinal fold. NPY were distributed mainly in the anterior part and posterior part of anterior intestine at a density of 18.7 cells/mm^2 and 26.3 cells/mm^2, respectively. But only a few of NPY cells were found in the midgut and posterior intestine, at a density of 5.1 cells/mm^2 and 5.5 cells/mm^2, respectively. The highest distribution density of β-endorphin, about 31.5cells/mm^2, was observed in the posterior intestine, and then, the density decreased sequentially from anterior intestine to midgut, at a density of 26.9, 11.6and 9.1 cells/mm^2, respectively.

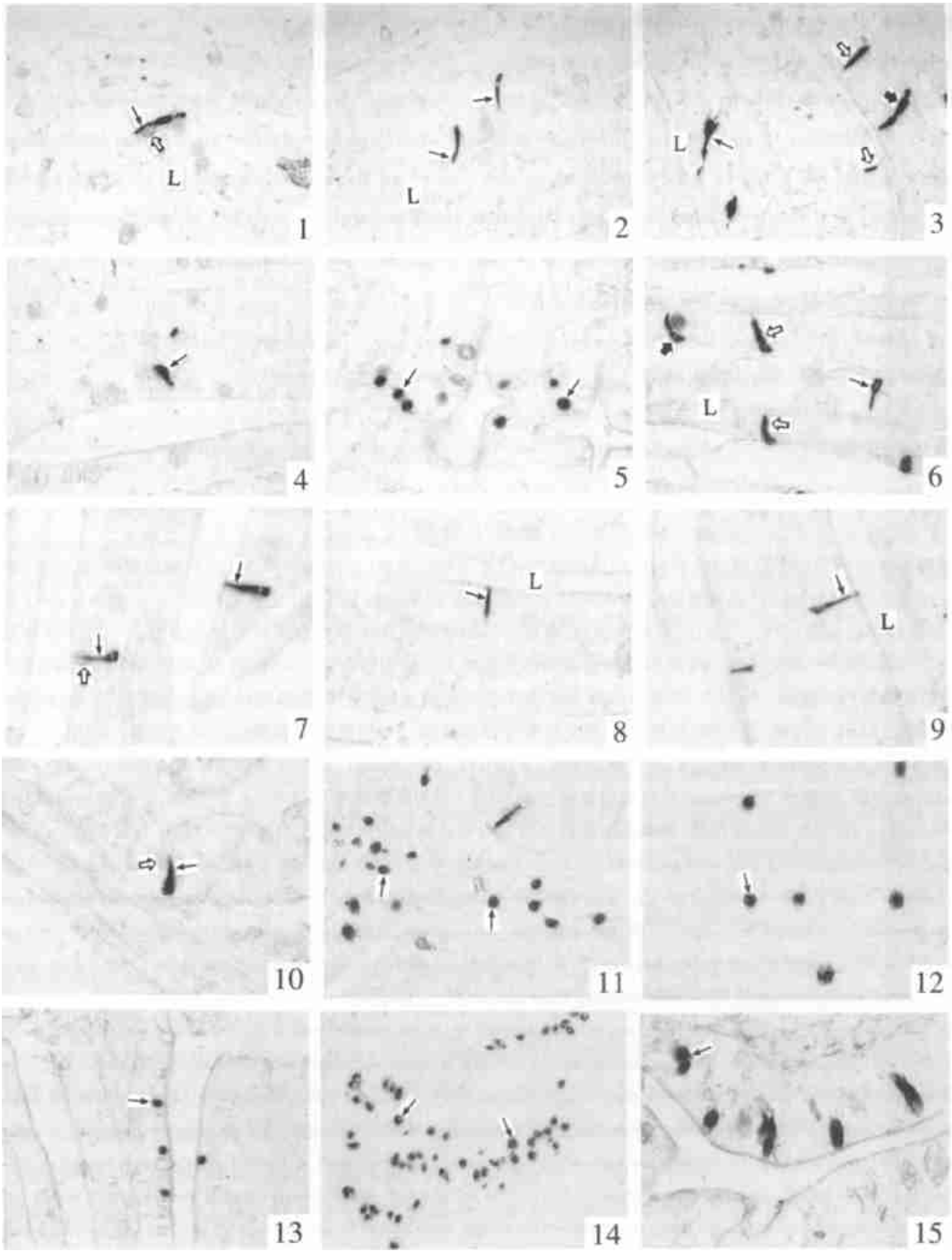
The morphological and distributional characters of NPY and β-EP immunoreactive cells in grey mullet intestine were similar to those in other teleost fishes (Fang et al., 1991; Al-Mahrouki and Youson, 1998). Their histological characters were similar to those described by Pand and Fang (1995). They were distributed scatterly in a single cell style between the epidemic cells and cup-shaped cells at intestinal mucosa. According to the distributional character and morphological type of NPY immunoreactive cells in the grey mullet intestine, especially the highest distributional density in anterior intestine and sparse distribution in midgut and posterior intestine and long cytoplasmic protrusion extending to the gut lumen. It is suggested that NPY might partly involved in the control of the secretion activity of other endocrine cells and gland cell in intestine, and the coordination of the function of intestine and the stability of microenvironment. On the other hand, the distribution of the two peptides in the intestine of grey mullet may provides basis for their evolutional conservation.

Key words: *Mugil cephalus*; Neuropeptide Y; β-endorphin; Immunocytochemistry

图版Ⅰ 说明

1. 在前肠前段, 长梭形 NPY 样免疫阳性细胞的突起(→)伸向肠腔(L)且与邻近细胞(⇨)接触, × 120; 2. 在前肠前段, 长梭形 NPY 样免疫阳性细胞伸出长突起(→)至肠腔(L), × 120; 3. 在前肠前段, 可见不同形态的 NPY 样免疫活性细胞, 长或短柱形(⇨)、烧瓶形(→)和葫芦形(➡), × 120; 4. 锥形 NPY 样免疫阳性细胞(→), × 120; 5. 在前肠前段, 卵圆形或椭圆形的 NPY 样免疫阳性封闭型细胞(→)位于肠褶基部, × 120; 6. 在前肠后段, 柱形(⇨)、烧瓶形(→)和葫芦形(➡)的 NPY 样免疫阳性细胞的突起伸向肠腔(L), × 120; 7. 长柱形 NPY 样免疫阳性细胞的突起(→)伸向肠腔(L)且与邻近细胞(⇨)接触, × 120; 8. 长梭形 NPY 样免疫阳性细胞的突起(→)伸向肠腔(L), × 120; 9. 在中肠, 柱形 NPY 样免疫阳性细胞突起(→)伸向肠腔(L), × 120; 10. 在中肠, 柱形 NPY 样免疫阳性细胞突起(→)伸至邻近细胞(⇨)与之接触, × 120; 11. 在前肠前段, 许多卵圆形或椭圆形的β-内啡肽样免疫阳性细胞(→)位于肠褶基部, × 120; 12. 在前肠后段, 卵圆形或椭圆形的β-内啡肽样免疫阳性细胞(→)位于肠褶中部, × 120; 13. 在中肠, 卵圆形β-内啡肽样免疫阳性封闭型细胞(→)位于肠褶后部, × 120; 14. 在后肠, 许多β-内啡肽样免疫阳性封闭型细胞(→)位于肠褶基部, × 120; 15. 在前肠前段, 卵圆形或椭圆形的β-内啡肽样免疫阳性封闭型细胞(→)位于肠褶基部, × 120

1. Long shuttle-shaped NPY-immunoreactve cells were located in the anterior segment of anterior intestine in *Mugil cephalus*, Protrusion(→) extended to intestinal lumen(L) and touched with adjacent cell. × 120; 2. Long shuttle-shaped NPY-immunoreactve cells in the anterior segment of anterior intestine extended long protrusion(→)to intestinal lumen(L). × 120; 3. Polymorphic NPY-immunoreactve cells of long or short column-shaped(⇨), flask-shaped(→) and calabash-shaped(➡), were seen in the anterior segment of anterior intestine. × 120; 4. Pyramid-shaped NPY-immunopositive cell(arrow). × 120; 5. In the anterior segment of anterior intestine, showing oval or ellipsis-shaped NPY-immunoreactive close type cells(→) located in the basic part of fold. × 120; 6. The protrusions of NPY-immunopositive cells in column-shaped(⇨), flask-shaped(→) and calabash-shaped(➡) in the posterior part of anterior intestine extended to intestinal lumen(L). × 120; 7. The protrusion(→) of long column-shaped NPY-immunoreactive cell extended to intestinal lumen(L) and touched with adjacent cell(⇨). × 120; 8. The protrusion(→) of long shuttle-shaped NPY-immunoreactive extended to intestinal lumen(L). × 120; 9. The protrusion (→) of column-shaped NPY-immunoreactive in midgut extended to intestinal lumen(L). × 120; 10. The protrusion(→) of column-shaped NPY-immunoreactive in midgut extended to and touched with adjacent cell. × 120; 11. Many oval or ellipsis-shaped β-endorphin-immunoreactive cells in the anterior segment of anterior intestine were located in basic part of intestine fold. × 120; 12. Oval or ellipsi-shaped β-endorphin-immunoreactive cells (→) in the posterior part of anterior intestine were located in middle part of intestine fold. × 120; 13. Oval-shaped β-endorphin-immunoreactive close type cells(→) in midgut were located in posterior part of intestine fold. @120; 14. Many β-endorphin-immunoreactive close type cells(y) in posterior intestine were located in basic part of intestine fold. @120; 15. Oval or ellipsis-shaped β-endorphin-immunoreactive cells(y) in the anterior segment of anterior intestine were located in basic part of intestine fold. @120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