

文章编号:1000-0615(2003)06-0513-06

日本鳗鲡胃肠胰内分泌细胞的鉴别与定位

林树根, 吴德峰, 王寿昆

(福建农林大学动物科学学院, 福建 福州 350002)

摘要:应用过氧化物酶标记的链霉亲和素(streptavidin-peroxidase 简称 S-P 法)免疫组织化学技术,用抗 5-羟色胺、生长抑素、胃泌素、 β -内啡肽、胰高血糖素抗体对日本鳗鲡的胃肠胰内分泌细胞进行研究。结果表明:日本鳗鲡胃肠道各段均存在 5-HT 和 Som 免疫活性内分泌细胞。Gas 细胞分布于胃体部、幽门部和前肠、中肠;胃贲门部和后肠未检出 Gas 细胞。 β -内啡肽、Glu 细胞在胃肠道各段均未检出。在胰腺中均鉴别出 Glu 细胞和 Som 细胞。

关键词:日本鳗鲡;胃肠胰系统;内分泌细胞;免疫组织化学反应

中图分类号:S917 **文献标识码:**A

Location and identification of endocrine cells in the gastro-entero-pancreas of *Anguilla japonicus*

LIN Shu-gen, WU De-feng, WANG Shou-kun

(College of Animal Science, Fujian Agriculture and Forestry University, Fuzhou 350002, China)

Abstract: With immunohistochemistry method, the endocrine cells in the gastro-entero-pancreas of eels, *Anguilla japonicus* were located and identified. Using 5-hydroxytryptamine(5-HT), somatostatin (Som), gastrin(Gas), β -endorphine(END) and glucagon(Glu) antisera, the endocrine cells in the gastro-entero-pancreas of the eel, *A. japonicus* were studied with an immunohistochemical technique of streptavidin labelled peroxidase. The weight of eels, coming from aquafarm, Fuqing, Fujian Province of China, was about 400g. The tissue section of the cardia, gastric fundus, pylorus, foregut, midgut, hindgut and pancreatic gland of the four eels were prepared. The concentrations of the antiserum of Glu, Gas, Som, 5-HT and END were 1:50, 1:1000, 1:100, 1:50 and 1:500 respectively. The major staining process was as follows: 1. to deparaffinize and rehydrate tissue section, and wash 2 times in buffer; 2. to reduce nonspecific background staining due to endogenous peroxidase, incubate the slide in 3% hydrogen peroxide block for 10-15 minutes, and wash 4 times in buffer; 3. to block nonspecific background staining, incubate for 10 minutes in buffers containing 10% normal goat serum at room temperature; 4. to apply primary antibody and incubate for one night at 4°C; 5. to incubate with biotin anti-mouse/rabbit IgG for 10 min at 37°C; 6. to incubate with streptavidin-peroxidase for 10 min at 37°C; 7. to incubate with DAB for 10 min at room temperature. The result showed that there is distribution of 5-HT and Som immune active endocrine cells in every part of digestive tract. Gas cells are distributed in the gastric fundus,

收稿日期:2002-12-09

作者简介:林树根(1950-),男,福建龙海人,副教授,主要从事动物组织学研究。E-mail:lin1950@sina.com

pylorus, foregut and midgut, and not found in cardia as well as hindgut. β -endorphine and Glu cells were not found in every part of digestive tract either. But Glu and Som cells were identified in the pancreatic glands. The densities of 5-HT, Som and Gas cells in the pylorus were highest. The shapes of 5-HT, Som and Gas cells were shuttle, triangle shape etc in the mucous epithelium of the digestive tract of the eels, there were longer cellular protuberance in the cells which could reach gastro-entero antrum and the cells were opening type cells. There were a small number of the cells of 5-HT, Som and Gas in the lamina propria of gastro-entero tract, there was no cellular protuberance in the cells and the cells were occlude type cells. In this paper, the morphologic characteristics of endocrine cells of gastro-entero-pancreatic system in *A. japonicus* are described and their distribution feature is discussed.

Key words: *Anguilla japonicus*; gastro-entero-pancreatic system; endocrine cell; immunohistochemical reaction

近20年来,国内外学者已对无胃真骨鱼类和有胃真骨鱼类消化道和胰腺中的部分激素,利用免疫细胞化学技术中抗原抗体反应的特异性和不可逆性准确地进行定位和鉴别,在鱼的消化系统中鉴别出11种散在的内分泌细胞^[1-15]。对多种鱼类的比较研究表明:鱼类胃肠胰中的内分泌细胞的种类和分布是非常复杂的,充分显示内分泌细胞在鱼类消化道分布的多样性。本研究用5种抗哺乳动物血清对日本鳗鲡胃肠胰内分泌细胞进行鉴别和定位,进一步丰富鱼类消化道比较内分泌学资料,为鱼类组织学和消化生理研究等有关学科注入新的概念和内容。

1 材料和方法

1.1 材料

日本鳗鲡4尾,每尾重约400g左右,取自福清渔溪养鳗场,断头放血,剖开腹腔,自下列消化道各段取材:胃贲门部、胃体部、胃幽门部、前肠、中肠、后肠和胰腺。用无水冰醋酸的Bouin氏液固定24h,常规脱水透明、石蜡包埋,切片厚度6 μ m,贴附于经多聚赖氨酸处理的玻片上,40 $^{\circ}$ C烘干24h。

1.2 主要试剂及药品

研究使用的胰高血糖素(Glu)、胃泌素(Gas)、生长抑素(Som)和5-羟色胺(5-HT)4种抗血清的工作浓度分别为1:50、1:1000、1:100和1:50。即用型免疫组织化学S-P试剂盒和以上一抗均购于福州迈新生物技术有限公司。兔抗 β -内啡肽(END)血清,工作浓度1:500,购于武汉博士德公司。

1.3 S-P的主要步骤

常规石蜡切片脱蜡至水。①3% H₂O₂ 室温孵育5min,消除内源性过氧化物酶的活性;②5%~10%正常山羊血清室温中孵育10min,消除非特异性抗体;③第一抗体4 $^{\circ}$ C过夜;④生物素标记的羊抗兔IgG血清37 $^{\circ}$ C孵育10min;⑤辣根过氧化物酶标记的链霉亲和素37 $^{\circ}$ C孵育10min;⑥3'-3'二氨基联苯二胺盐酸盐(DAB)室温中显示10min;⑦对照片以相应部位的连续切片并用PBS取代一抗血清,其余步骤均按上述方法同步进行。

1.4 观察、计数

在Olympus BH-2显微镜下观察、鉴别、计数、显微摄影。对每个取材部位随机取10个视野计数,计算出每平方毫米内分泌细胞数,再将所有数据进行统计,算出平均值($\bar{X} \pm SD$),以此表示内分泌细胞的分布密度。

2 结果

日本鳗鲡胃肠胰中5种内分泌细胞的定位情况及分布密度见表1。

表 1 日本鳗鲡胃肠胰中 5 种内分泌细胞的分布和密度

Tab.1 Distribution of 5 kinds of endocrine cell in the gastro-entero-pancreatic system *A. japonica*

内分泌细胞 endocrine cell	Cell·mm ⁻²						
	贲门 cardia	胃底 fundus	幽门 pylorus	前肠 foregut	中肠 midgut	后肠 hindgut	胰岛 pancreatic island
5-羟色胺(5-HT)	10±3.5	13±3	27±5.5	8±3	14±4.5	21±4	-
生长抑素(Som)	9±3	22±4.5	26±4	10±3.5	5±2	3±2	63±13
胃泌素(Gas)	-	6±2.5	31±6.5	5±3	偶见 little	-	-
β-内啡肽(END)	-	-	-	-	-	-	-
胰高血糖素(Glu)	-	-	-	-	-	-	92±25

注:“-”表示阴性反应

Notes:“-” means negative reaction

5-羟色胺(5-HT)细胞:5-HT细胞分布于胃粘膜上皮细胞之间,细胞胞体较大,细胞呈纺锤形、三角形、长梭形,可见一胞突从上皮细胞之间伸向胃腔(图版-1,2);在消化道各段均有分布,胃贲门部、胃体部分布较少,胃幽门部分布较密集(图版-3);5-HT细胞在前肠分布最少,中肠、后肠呈逐渐增多的趋势。胃贲门部和胃体部的胃腺未见5-HT细胞分布,胃幽门部的幽门腺腺泡上皮细胞之间有5-HT细胞,细胞形态同胃粘膜上皮的5-HT细胞大体相同,游离端突起伸达腺腔(图版-3);少量分布于胃固有层结缔组织中,细胞体积较小,呈球形或不规则形(图版-2)。肠道5-HT细胞主要位于粘膜上皮细胞之间和固有膜结缔组织中,胞体较小,细胞呈长柱形、锤形、球形和不规则形,在肠上皮细胞之间的5-HT细胞,可见有的胞突直接开口于肠腔(图版-4,5)。胰腺未检出5-HT阳性细胞。

生长抑素(Som)细胞:胃体部和胃幽门部分布密度较高(图版-7,8),胃贲门部、肠管各段分布密度低,尤其是后肠最少。Som细胞在胃主要分布于胃粘膜上皮细胞之间,细胞呈锤形、三角形和梭形,游离端也有突起伸向胃腔(图版-6,7);胃腺只有幽门腺腺泡细胞之间有Som细胞分布,细胞形态与胃粘膜上皮的Som细胞相同,游离端也有突起伸达腺腔(图版-8)。肠道Som细胞分布于粘膜上皮细胞之间和固有膜结缔组织中,肠粘膜上皮细胞之间Som细胞多呈长梭形,胞突伸向肠腔(图版-9),固有膜中的Som细胞多为球形或不规则形。Som细胞分布于胰腺的胰岛,呈分散分布,细胞形状不规则,胞突相连似网状(图版-10)。

胃泌素(Gas)细胞:分布于胃体部、幽门部和前肠,其中以幽门部分布最为密集(图版-12),中肠偶见Gas细胞分布,而胃贲门部和后肠未见Gas细胞分布。Gas细胞在胃体部分布于粘膜细胞之间,细胞为梭形、三角形,游离端突起伸向胃腔(图版-11);少量分布于固有膜结缔组织中,细胞为球形,未见突起。胃幽门部Gas细胞分布于粘膜上皮细胞之间与幽门腺腺泡细胞之间,细胞呈梭形、三角锥形、梨形、游离端的突起分别伸达胃腔和腺腔(图版-12,13);固有膜有少量Gas细胞,为卵圆形、不规则形。肠道Gas细胞位于肠粘膜上皮细胞之间,细胞有长条形、梭形,游离端有突起伸达肠腔(图版-14);固有膜Gas细胞很少,细胞形态不规则。

胰高血糖素(Glu)细胞:分布于胰腺的胰岛,有分散分布,也有成团分布。细胞形状呈圆形、卵圆形和不规则形(图版-15),胰高血糖(Glu)细胞在消化道各段均为阴性。β-内啡肽(END)细胞在消化道和胰腺均未检出。所有对照片均为阴性反应。

3 讨论

鱼类5-HT细胞在胃肠道的分布情况,有胃真骨鱼和无胃真骨鱼存在很大差别。5-HT细胞在8种无胃鱼的肠中均未发现^[5,9,12],而在有胃鱼的消化道均有分布^[10,11,13,14]。本研究的日本鳗鲡胃肠道均有5-HT细胞分布,与上述有胃鱼相似。日本鳗鲡5-HT细胞以胃幽门部密度最高,前肠密度最低,中肠、后肠阳性细胞密度又上升,后肠达到较高密度,与其他作者报道的略有不同^[10,11,13,14]。幽门部含大量5-HT细胞分泌5-HT,能刺激胃肠粘液分泌,平滑肌收缩和血管扩张,对消化功能有很强的调节作用^[15];而后肠主要功能为排便,日本鳗鲡5-HT细胞在后肠密度较高,这种分泌型可能与5-HT调

节肠道运动以利于排便的功能是一致的。

Som 细胞在有胃鱼消化道的分布已有一些报道^[10,13,14]。但分布型亦有差别,河鲈(*Perca fluviatilis*)和云斑鲮(*Ameiurus nebulosus*)、短盖巨脂鲤(*Colossoma brachypomun*)3种鱼,Som 细胞仅存在胃粘膜,肠道中未见到^[1,10];尼罗罗非鱼(*Tilapia nilotica*)、鳊(*Siniperca chuatsi*)整个胃肠道均有 Som 细胞分布^[10,14],但也有报道有胃鱼鲇(*Silurus asotus*)、乌鳢(*Channa argus*)和大口黑鲈(*Micropterus salmoides*)消化道粘膜均未见到 Som 内分泌细胞^[12]。

日本鳊鲮胃肠道均有较多 Som 细胞分布,以胃体部和胃幽门部最多,且分布于粘膜上皮细胞之间和胃幽门腺腺泡细胞之间,多数细胞有一突起伸向胃腔或腺腔,呈开放型。当胃内 pH 值下降时,Som 细胞受刺激而释放生长抑素,从而抑制 Gas 细胞释放胃泌素,降低壁细胞的胃酸分泌等^[16]。显然,日本鳊鲮 Som 细胞分布特点,对胃肠功能的调节具有重要意义。

Gas 细胞在鱼类消化道分布的研究报道较多,先前研究已证实 9 种鲤科鱼类的肠道中,Gas 细胞大多在前肠前段(肠球部)分布^[3,4,6,8,12];在有胃鱼鳊鱼、尼罗罗非鱼、短盖巨脂鲤、鲇和大口黑鲈胃中均未发现 Gas 细胞^[10,11,14]。而乌鳢的胃体部和幽门部有较多 Gas 细胞分布^[11]。本研究在日本鳊鲮胃体部、幽门部、前肠和中肠均有 Gas 细胞分布,根据已研究的结果,说明鱼类胃肠道 Gas 细胞分布情况存在很大差异。Gas 细胞分泌胃泌素主要生理功能是刺激胃酸、胃蛋白酶分泌、胃窦收缩,增加胃粘膜血流量^[17]。日本鳊鲮 Gas 细胞胃幽门部密度最大,前肠次之,中肠偶见,后肠没有,胃、前肠是主要的消化吸收场所,需依靠胃泌素来调节消化吸收功能。因此,Gas 细胞分布型与其功能是相一致的。

根据形态学特征,消化道内分泌细胞分为两类:一类是开放型细胞,该类细胞的突出特点是都有较长的胞突通向消化腔;另一类是闭合型细胞,该类细胞多呈圆形,与消化腔无直接联系^[8]。日本鳊鲮 5-HT 细胞、Som 细胞和 Gas 细胞,大多数呈梭形、纺锤形和三角形,细胞都可见较长的胞突伸向胃肠腔或胃腺腔,显然属于开放型细胞。这类细胞可通过其较长的胞突直接感受消化腔内内容物的刺激而分泌,从而调节胃肠功能的活动。而日本鳊鲮 5-HT 细胞、Som 细胞和 Gas 细胞,少量分布于胃肠粘膜固有层,细胞呈圆形或不规则形,很少见有胞突,与胃肠腔、腺腔无直接联系,属于闭合型细胞。这类细胞可能是通过感受局部组织内环境变化和消化腔内容物压力的刺激而分泌^[18]。但也有人认为这类细胞是由于切片所致而缺少胞突^[6],这还有待今后深入研究。

参考文献:

- [1] Noailiac-Depeyre J, Hollande E. Evidence for somatostatin, gastrin and pancreatic polypeptide-like substances in the mucosa cells of the gut in fishes with and without stomach[J]. Cell Tiss Res, 1981,216:193-203.
- [2] Rombout J W, Reinecke M. Immunohistochemical localization of (neuro-) peptide hormones in endocrine cells and nerves of the gut of a stomachless teleost fish, *Barbus conchoni* (Cyprinidae) [J]. Cell Tiss Res, 1984, 237:57-65.
- [3] Abad M E, Binkhorst F M P, Elbal M T. A comparative immunocytochemical study of the gastro entero pancreatic (GEP) endocrine system in a stomachless and a stomach-containing teleost[J]. Gen Comp Endocrinol, 1987,66:123-136.
- [4] Pan Q S, Fang Z P. The immunohistochemical localization and comparison of gastrin-immunoreactive cells in the intestinal tract of four carps species [J]. J Huazhong Agric Univ, 1988,7(3):238-242. [潘黔生,方之平.四种鲤科鱼类肠道中胃泌素免疫活性细胞的免疫组织化学定位及比较[J].华中农业大学学报,1988,7(3):238-242.]
- [5] Fang Z P, Junzo Yamada, Pan Q S. Immunohistochemical identification and localization of endocrine cells in the intestinal mucosa of common carp and black carp[J]. Acta Hydrobiol Sin, 1991,15(3):212-219. [方之平,山田纯三,潘黔生,等.鲤、青鱼肠粘膜内分泌细胞的免疫组织化学鉴别和定位[J].水生生物学报,1991,15(3):212-219.]
- [6] Pan Q S, Fang Z P. An immunocytochemical study of endocrine cell in the gut of a stomachless teleost fish, grass carp, Cyprinidae [J]. Cell Transplantation, 1993,2(5):419-427.
- [7] Pan Q S, Fang Z P, Fan Q X, et al. immunohistochemical localization and morphological study on G cell in the intestine of *Hypophthalmichthys molitrix*, *Aristichthys nobilis*, *Carassius auratus gibelio* and *Megalobrama amblycephala* [J]. Acta Zool Sin, 1995,41(2):167-172. [潘黔生,方之平,樊启学,等.鲢、鳊、银鲫和团头鲂肠道 G 细胞定位与免疫组化研究[J].动物学报,1995,41(2):167-172.]
- [8] Pan Q S, Fang Z P. Present progress in the study of the APUD cells in gastro-entero-pancreatic endocrine system of the fishes [J]. Acta Hydrobiol Sin, 1995, 13(3):275-282. [潘黔生,方之平.鱼类胃肠膜内分泌系统 APUD 细胞研究的现状[J].水生生物学报,1995,19(3):275-

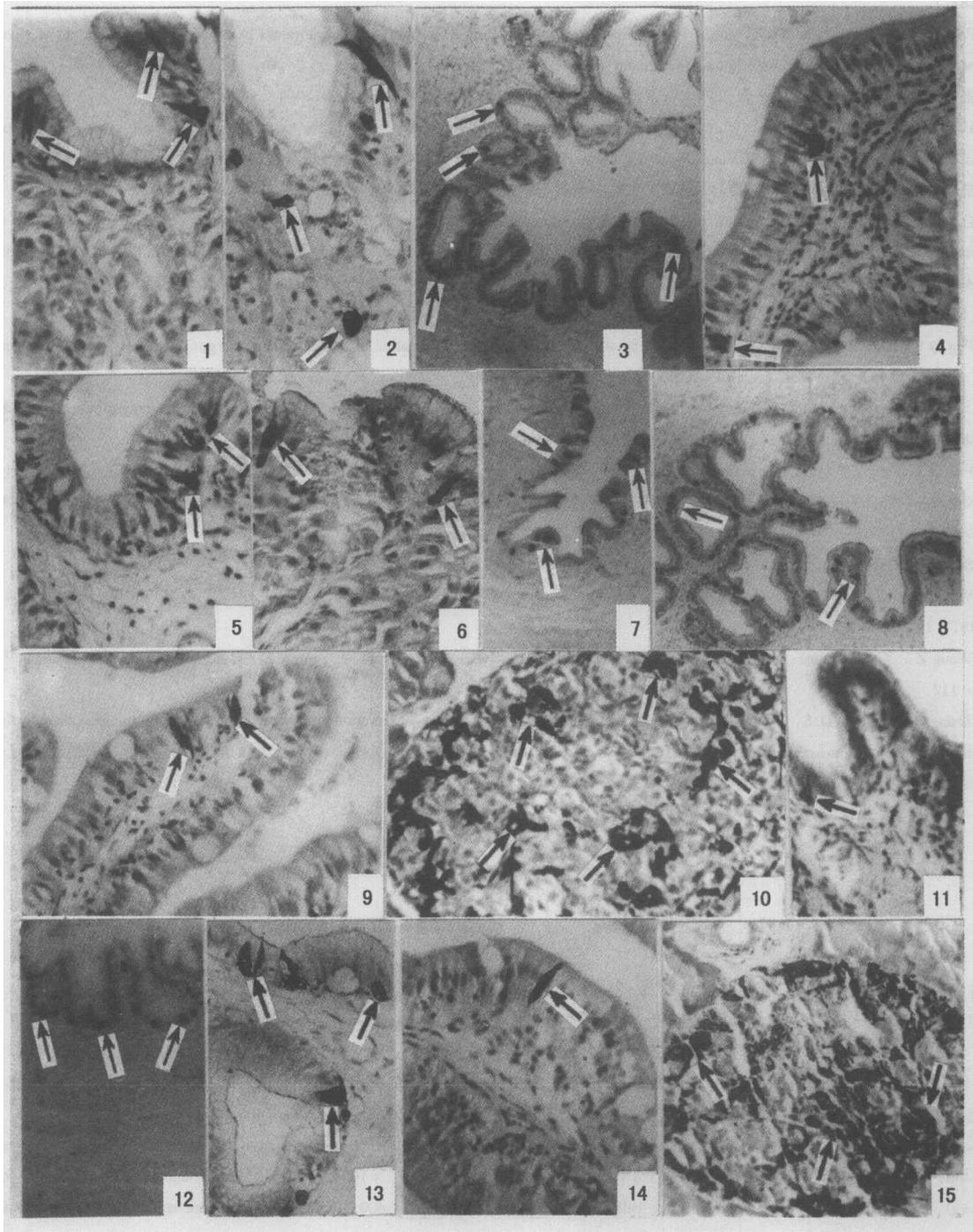
282.]

- [9] Pan Q S, Fang Z P, Zhao Y X, *et al.* Immunohistochemical studies of 3 kinds of peptide hormones in endocrine cells of the gut of silver carp, bighead, silver crucian carp and blunt-snout bream[J]. *Acta Hydrobiol Sin*, 1996,20(4):312-316. [潘黔生,方之平,赵雅心,等. 鲢、鳊、银鲫和团头鲂肠道内分泌细胞三种肽激素的免疫组化研究[J]. *水生生物学报*, 1996,20(4):312-316.]
- [10] Fang Z P, Pan Q S, Nie X Y, *et al.* Localization and identification of endocrine cells in the gastro-entero-pancreatic system of two species of stomachcontaining teleosts[J]. *J Huazhong Agric Univ*, 1997,16(6):594-598. [方之平,潘黔生,聂秀云,等. 两种有胃真骨鱼胃肠胰系统中内分泌细胞的鉴别与定位[J]. *华中农业大学学报*, 1997,16(6):594-598.]
- [11] Fang Z P, Pan Q S, Zhao Y X. Local ization and comparison of endocrine cells in the digestive tract mucosa of largemouth bass, northern snakehead and oriental sheatfish[A]. *China Animal Science Research(Collected papers on the 65th anniversary of Animal Society in china)*[C]. Beijing:Forestry Press, 1999.1031-1035. [方之平,潘黔生,赵雅心,等. 鲈、乌鳢和大口黑鲈消化道粘膜中内分泌细胞的定位及比较[M]. *中国动物科学研究(中国动物学会成立65周年论文集)*. 北京:林业出版社,1999.1031-1035.]
- [12] Pan Q S, Fang Z P, Zhao Y X. Immunocytochemical identification and localization of APUD cells in the gut of seven stomachless teleost fishes [J]. *World Journal of Gastroenterology*, 2000,6(1):96-101.
- [13] Pan Q S, Fang Z P, Huang F J. Identification, localization and morphology of APUD cells in gastro-entero-pancreatic system of stomachcontaining teleosts [J]. *World Journal of Gastroenterology*, 2000,6(6):842-847.
- [14] Pan Q S, Fang Z P. Immunocytochemical evidence for two hormones in the gastrointestinal endocrine cells of mandarin fish [J]. *Acta Hydrobiol Sin*, 2001,25(6):565-570. [潘黔生,方之平. 几种激素在鳊胃肠道内分泌细胞中存在的免疫细胞化学证据[J]. *水生生物学报*, 2001,25(6):565-570.]
- [15] Solcia E, Capella C, Vassallo G, *et al.* Endocrine cells of the gastric mucosa[J]. *Int Rev Cytol*, 42:223-286.
- [16] Wang Z J. Gut Hormone: Somatostatin[M]. Beijing: Science Press, 1985. [王志均. 胃肠激素: 生长抑素[M]. 北京: 科学出版社, 1985. 197-199.]
- [17] Wang Z J. Gut hormone: gastrin[M]. Beijing: Science Press, 1985. 104-112. [王志均. 胃肠激素: 胃素[M]. 北京: 科学出版社, 1985. 104-112.]
- [18] Zhang S Z, Chen D S, Zhang Z Q, *et al.* Distribution and morphology observation of 5-hydroxytryptamine immunoreactive cells in the digestive tract of *Pachytriton labiatum*[J]. *Chinese J Zool*, 2001,36(3):13-16. [张盛周, 陈冬生, 张志强, 等. 无斑肥螈消化道五羟色胺免疫活性细胞的分布与形态学观察[J]. *动物学杂志*, 2001,36(3):13-16.]

图版说明

Explanation of Plate

1. 胃贲门部 5-羟色胺细胞(箭头), × 400; 2. 胃底部 5-羟色胺细胞(箭头), × 400; 3. 胃幽门部 5-羟色胺细胞(箭头), × 100; 4. 前肠 5-羟色胺细胞(箭头), × 400; 5. 后肠 5-羟色胺细胞(箭头), × 400; 6. 胃贲门部生长抑素细胞(箭头), × 400; 7. 胃底部生长抑素细胞(箭头), × 100; 8. 胃幽门部生长抑素细胞(箭头), × 100; 9. 前肠生长抑素细胞(箭头), × 400; 10. 胰岛生长抑素细胞(箭头), × 400; 11. 胃底部胃泌素细胞(箭头), × 400; 12. 胃幽门部胃泌素细胞(箭头), × 100; 13. 胃幽门部胃泌素细胞(箭头), × 400; 14. 前肠胃泌素细胞(箭头), × 400; 15. 胰岛胰高血糖素细胞(箭头), × 400
1. 5-HT immunoreactive cells in the stomach cardiacus (arrows), × 400; 2. 5-HT immunoreactive cells in the stomach fundus (arrows), × 400; 3. 5-HT immunoreactive cells in the stomach pyloricus (arrows), × 100; 4. 5-HT immunoreactive cells in the foregut (arrows), × 400; 5. 5-HT immunoreactive cells in the hindgut (arrows), × 400; 6. Some immunoreactive cells in the stomach cardiacus (arrows), × 400; 7. Some immunoreactive cells in the stomach fundus (arrows), × 100; 8. Some immunoreactive cells in the stomach pyloricus (arrows), × 100; 9. Some immunoreactive cells in the foregut (arrows), × 400; 10. Some immunoreactive cells in the pancreatic island, (arrows), × 400; 11. Gas immunoreactive cells in the stomach fundus (arrows), 400; 12. Gas immunoreactive cells in the stomach pyloricus (arrows), × 100; 13. Gas immunoreactive cells in the stomach pyloricus (arrows), × 400; 14. Gas immunoreactive cells in the foregut (arrows), × 400; 15. Glu immunoreactive cells in the pancreatic island (arrows), × 400



图版 Plate