# Characterization of enterochromaffin cells isolated from the rat ileum.

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Enterochromaffin (EC) cells are distributed throughout the digestive epithelium. This cell type releases serotonin besides a variety of peptides and hormones, thereby being a key messenger for intestinal motility as well as secretion. Studies using highly enriched, isolated EC cells from rat ileum now reveal a large spectrum of receptors specifically expressed on this cell type, for example β-adrenergic, muscarinic as well as GABA-ergic receptors. Interestingly, also EC cells seem to express several bitter taste and olfactory receptors, which might shed a light on a physiological function as a 'sentinel cell' in the gut. Current studies determine the response of these enriched cells towards receptor antagonists as well as antagonists, offering a large perspective to treat patients with motility disorders, diarrhoea or constipation. Gastric EC-like cells, in contrast, have been characterised since already ten years. This cell type releases histamine as a paracrine stimulant, thereby controlling the peripheral regulation of acid secretion. ECL cells respond to treatment with gastrin as well as PACAP with histamine release; somatostatin and galanin inhibit the release. So far, there is a lot of information regarding the physiological function; ECL cells, however, have not become a target for pharmacological therapy. Microarray techniques are currently being performed to determine key factors during the malignant transformation of these tumors.

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**Enterochromaffin-like (ECL) cells: Lessons from the past.** ECL cells are histamine-containing neuroendocrine cells located in the lower third of the acid-secreting gastric epithelium[1,2]. They constitute more then 30% of the neuroendocrine cell population of the gastric mucosa and are important for the peripheral regulation of gastric acid secretion. In response to gastrin and PACAP they release histamine in a calcium-dependent manner thereby stimulating acid secretion from parietal cells. Storage of histamine in secretory vesicles is dependent on transport via the vesicular monoamine transporter-2 (VMAT-2). Hypergastrinemia leads to proliferation and tumor development of ECL cells. These tumors infiltrate nearby structures but never metastatize. ECL cells can be isolated and highly enriched from the gastric mucosa. Previous studies have characterized the presence of certain exocytotic and endocytotic proteins specifically in ECL as well as other neuroendocrine cells, thereby serving as marker genes to identify the characteristic parameters during mircroarry techniques[3,4]. Thus, cell biology studies have served as a basis to determine changes during inflammatory or malignant transformations in the gut.

**Enterochromaffin (EC) cells.** EC cells are polarized neuroendocrine cells of the gastrointestinal epithelium, which synthesize, store and release serotonin (5-HT) as well as a variety of peptides such as guanylin[5,6]. They accumulate 5-HT in secretory vesicles via VMAT-1[7,8]. In vivo, 5-HT release is sensitive to mechanical stimulation or acidification of the gut lumen[9,10]. 5-HT regulates intestinal motility and transport in paracrine/endocrine and neuronal ways[11]. Activation of 5-HT receptors on intestinal crypt cells leads to chloride secretion[12]. The importance of 5-HT receptors on enteric nerves varies among intestinal regions and species[13,14]. Binding of 5-HT to the G-protein coupled 5-HT4 receptor on intrinsic neurons elevates intracellular cAMP levels and inhibits voltage gated $K^+$ channels, leading to depolarization and hence increased excitability. 5-HT4 receptors also appear to be important for the initiation of peristaltic reflexes. Furthermore, 5-HT is thought to play a role in generation of some diarrheal states caused by bacterial toxins [15,16] and several pathological conditions such as vomiting, diarrhea after radio- or chemotherapy[17,18] and irritable bowel syndrome[19,20].

A large number of stimulatory and inhibitory receptors have already been postulated as being present on EC cells [21]. However, the actual target and hence mechanism of action of most of the drugs used in intestinal therapy is not clear since only heterocellular tissue or organ preparations have been used[21,22]. The release of serotonin is thought to be calcium-dependent, suggesting the involvement of receptor-operated or voltage-dependent calcium channels, although a cellular attribution is difficult in the crypt preparations investigated[23]. An enriched preparation to investigate functions of and potential pharmacologic targets on EC cells of the small intestine has not been available to date.

**Table 1: Enrichment of EC Cells from rat intestine as determined by Immunocytochemistry and serotonin content**

| | **Mucosal cells** | **EC Cells** |
|---|---|---|
| **5-HT positive cells (%)** | 12 ± 4 | 84 ± 6 |
| **SNAP-25 positive cells (%)** | 42 ± 4 | 86 ± 8 |
| **5-HT content ($pg/10^5$ cells)** | 0.5 – 1 | 6 – 12 |

**Isolation of enterochromaffin cells from the intestine.** To obtain an enriched fraction of isolated EC cells, a combination of enzymatic digestion, counterflow elutriation, density gradient centrifugation and short-term culture was applied. The protocol used the small size and high cellular density of EC cells and was a modification of the isolation procedure for gastric ECL cells[24]. With this procedure, EC cells could be enriched ~7fold compared to the crude cell suspension (MUC) based on serotonin immunocytochemistry (table 1). Most of the contaminating cells in the enriched fraction were identified as goblet cells by PAS staining. The high percentage of SNAP-25 positive cells in the unfractionated cells indicates the presence of other neuroendocrine cells. While most of the isolated cells excluded Trypan blue, a loss of cell integrity occurred within 24 h in culture. This loss of survival seems characteristic of isolated intestinal epithelial cells[25]. Serotonin content was significantly enhanced in isolated EC cells when compared to

unfractionated mucosal cells from the ileum, yielding a 7-12 fold enrichment (see table 1).

**Receptors present on EC cells.** RT-PCR and imuno-cytochemistry were performed on EC cells after the enrichment procedure to determine possible receptors present on these cells. As shown in table 2, both methods gave positive results for alpha2A and beta1 adrenoreceptors as well as muscarinic M3 and the GABA-A receptors. Furthermore, RT-PCR revealed the presence of RNA for alpha2B and beta2 adrenoreceptors. No positive signal was obtained for the GABA-B receptor. The presence of adreno- and muscarinic receptors in EC cells has been deduced by Racke et al. based on data using isolated porcine or guinea pig tissue strips[26-28]. Thus, the isolated and enriched cells largely confirm these previous data. In contrast to previous studies[29], no 5-HT3 and 5-HT4 receptors were detected on isolated EC cells.

**Table 2: Receptors expressed on EC cells**

| Receptor | Immunucytochemistry | RT-PCR | Affymetrix Gene chip |
|---|---|---|---|
| $\alpha_{2A}$ | + | + | + |
| $\alpha_{2B}$ | n.d. | + | + |
| $\beta_1$ | + | + | + |
| $\beta_2$ | n.d. | + | + |
| $M_3$ | + | + | + |
| GABA-A | + | + | + |
| GABA-B | n.d. | - | ? |
| 5-$HT_3$ | n.d. | - | - |
| 5-$HT_4$ | n.d. | - | - |

**Gene chip analysis.** To further investigate gene expression in isolated EC cells, the Affymetrix GeneChip Rat Genome RG-U34A array, which allows detection of 5467 genes of known function, was used. Several genes were identified which showed both a relatively high level of expression (400 or greater) as well as a more than 4-fold

higher expression level in the EC cell fraction as compared to unfractionated mucosal cells (table 3). Expression of components necessary for storage of serotonin in EC cells was as expected. For example, the vesicular monoamine transporter 1 (VMAT-1), a specific marker of EC cells, was present, but not VMAT-2 which is present in gastric ECL cells[7]. In addition, a sodium dependent neurotransmitter transporter[30] appears to be expressed in EC cells. Animals lacking the serotonin reuptake transporter (SERT) still have the ability to accumulate serotonin in EC cells[31], suggesting a possible involvement of this plasmalemmal transmitter transporter found here.

**Expected receptor expression on EC cells as determined by gene chip analysis.** A variety of receptor genes were detected that were both highly enriched (> 7-fold) and expressed at high levels. It appears that the cells in the preparation selectively express the $\alpha_{1C}$ adrenoreceptor, nicotinic acetylcholine receptors, the histamine-2 receptor, GABA-A receptor, metabotropic and ionotropic glutamate (AMPA R1 and NMDA receptors R1 and others). Most of these receptor subtypes were also identified using IC and RT-PCR, similar to results in previous works using intestinal strips[29]. In contrast, GABA-B receptors were detected only in Gene chips, indicating there may be a cross-reacting with sequences to related receptor sequences such as glutamate receptors. GABA-B receptors are heterodimeric complexes with numerous splice variants. The heterogeneity of GABA-B receptors is quite complex and not fully investigated in detail yet[32]. Presence of "metabotropic" GABA-B receptors was determined by immunohistochemistry here in previous works[33]; however, several receptor subtypes exist which may bind various antibodies. N-methyl-D-aspartate receptors (NMDARs) are usually present at many excitatory glutamate synapses in the central nervous system, displaying unique properties that depend on their subunit composition[34]. Although not confirmed by specific immunostaining, the clustering of these genes suggests the selective expression

**Table 3: Relative enrichment of EC cell mRNA expression in purified EC cells**

| | MUC (n=2) | EC (n=3) | x-fold |
|---|---|---|---|
| $\beta_2$ adrenergic receptor protein (RG20) | 14.3 | 212.4 | 14.9 |
| $\alpha_{2A}$ adrenergic receptor protein (promoter) | 189.2 | 897.0 | 4.7 |
| $\alpha_{1C}$ adrenergic receptor protein (promoter) | 32.4 | 677.4 | 20.9 |
| $\alpha_1$ adrenergic receptor | 34.9 | 155.3 | 4.5 |
| $\beta_2$ adrenergic receptor gene | 140.3 | 570.2 | 4.1 |
| $\beta_3$ adrenergic receptor (spliced version) | 118.9 | 696.5 | 5.9 |
| $M_1$ muscarinic receptor | 158.9 | 147.5 | 0.9 |
| $M_2$ muscarinic receptor | 131.0 | 440.1 | 3.4 |
| $M_3$ muscarinic receptor | 119.3 | 523.7 | 4.4 |
| $M_4$ muscarinic receptor | 229.1 | 460.6 | 2.0 |
| Nicotinic acetylcholine receptor $\alpha_6$ subunit | 683 | 607.1 | 8.9 |
| GABA-A receptor $\alpha_1$ subunit | 46.9 | 330.2 | 7.0 |
| GABA-A receptor $\beta_2$ subunit | 69.6 | 455.6 | 6.6 |
| GABA-A receptor $\beta_5$ subunit | 43.9 | 193.0 | 4.4 |
| GABA-A receptor $\delta_6$ subunit | 22.8 | 277.7 | 12.2 |
| GABA-A receptor $\gamma$ subunit | 172.8 | 1535.6 | 8.9 |
| GABA-A receptor $\rho_2$ subunit | 50.6 | 206.2 | 4.1 |
| GABA-B receptor 1c | 122.5 | 503.5 | 4.1 |
| GABA-B receptor 1d | 198.5 | 1178.8 | 5.9 |
| metabotropic glutamate receptor mGluR7 | 15.55 | 291.5 | 18.7 |
| glutamate receptor (GluR-B) | 22.3 | 345.3 | 15.5 |
| glutamate transporter | 35.0 | 238.3 | 6.8 |
| NMDA-R1 | 33.5 | 970.7 | 29.3 |
| NMDA-R2C | 198.2 | 991.2 | 5.0 |
| NMDA-R2D1 | 148.6 | 645.6 | 4.3 |
| NMDA-NR3 | 122.0 | 511.2 | 4.2 |
| AMPA GluR1 | 35.6 | 818.7 | 23.2 |
| olfactory OL1 | 84.1 | 535.4 | 6.4 |
| olfactory QIL-LD1 | 161.7 | 1274.4 | 7.9 |
| olfactory HGL-SL3 | 56.8 | 529.4 | 9.3 |
| olfactory HGL-SL2 | 81.0 | 533.4 | 6.6 |
| olfactory HVL-VN1 | 93.0 | 751.9 | 8.1 |
| olfactory receptor-like protein | 369.0 | 1622.1 | 4.4 |
| olfactory EVA-TN1 | 191.5 | 876.3 | 4.6 |
| olfactory SCR-D7 | 76.4 | 630.0 | 8.3 |
| olfactory SCR-D8 | 164.6 | 734.0 | 4.5 |
| olfactory receptor-like proteinSCR-D9 | 139.7 | 744.2 | 5.4 |
| putative pheromone receptor | 44.8 | 997.9 | 22.3 |
| putative pheromone receptor | 27.2 | 567.4 | 20.9 |
| putative pheromone receptor | 128.0 | 1139.5 | 8.9 |

of these receptors in EC cells. In addition, the metabotropic glutamate receptor subunit 4C appears to be related to EC cells. A possible effect of glutamate on EC cells may be related to the stimulation of serotonin secretion following neuronal stimulation.

A relative increase in mRNA was also detected for the muscarinic M3 and M5 acetylcholine receptor subtype and some subunits of the NMDA receptor. A high expression level and similar enrichment was also observed for the dopamine receptor D4. Most of the receptors demonstrated as present by gene chip analysis are not yet amenable to specific immunostaining, but the detection of many genes of several neuronal receptor families suggest that the cells in this EC cell enriched preparation have both neuronal and endocrine characteristics.

**Un-expected receptor and protein expression on EC cells.** Surprisingly, we also found clustering of several genes encoding olfactory and vomeronasal receptors. Such genes encoded olfactory SCR, HGL, HFL, QIL, and EVA receptor subtypes and pheromone receptors. The presence of these receptors was completely unexpected; however, the strong signal of several subtypes may suggest that some are selectively expressed in EC cells. It appears that EC cells may analyze chyme, similar to other intraepithelial sensors in the airway or blood system. The possibility exists that olfactory receptors may have a more general function in chemodetection similar to the situation observed in spermatids, taste buds and olfactory systems[35].

Besides the marker protein guanylin, secretin, VIP and galanin were detected as highly expressed and enriched in the EC cell fraction but other peptides were also present in the preparation such as vasopressin, secretogranin II and atrial natriuretic peptide (ANP). Vasopressin is a potent vasoconstrictor in the rat jejunum, enhances proliferation of mucosa cell lines and has been found previously in the gastrointestinal tract in nerve fibers and in enteroendocrine cells[36,37]. These findings may indicate that EC cells play an important role in blood flow and motility (via $V_1$ receptors in smooth muscle), resorption of electrolytes and fluid retention in the intestine. Secretogranin II, which is processed in the intestine to secretoneurin,

has previously been localized in D-cells of the human gut[38]. Secretoneurin has been described as a link to the immune system by inducing the migration of monocytes and leukocytes[38]. A possible function of ANP in the EC cell may be related to processes of electrolyte and water transport in the mucosa. Finally, factors involved in intracellular signaling were identified as highly expressed and enriched in the EC cell fraction. These include components participating in $IP_3$ metabolism and coupling via G-proteins ($IP_3$ binding proteins, inositol polyphosphate phosphatase, G protein coupled kinase 6). These components may participate in signal transduction after activation of olfactory or other receptors observed to be highly expressed in EC cells.

## SUMMARY

The current data generated by gene chip analysis suggest the presence of certain specific genes in EC cells; many of which appear to be new to this cell type, some have already been assigned to EC cells by immuncytochemistry. Additional studies are needed to determine the accuracy of the gene chip readout and to assign specific proteins to EC cells rather than other small neuroendocrine cells. Our novel approach therefore defines several potential proteins of interest in regulation of EC cell function. Continuation of these studies at the proteomic level will likely yield a better understanding of the biology of this important neuroendocrine cell.

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## REFERENCES

1. Prinz, C., et al., *The mechanism of histamine secretion from gastric enterochromaffin-like cells.* Am J Physiol, 1999. **277:**C845-C855.
2. Prinz, C., et al., *Physiology of gastric enterochromaffin-like cells.* Ann Rev Physiol, 2003.**65:**371-382.

3. Höhne-Zell, B., et al., *Functional importance of synaptobrevin and SNAP-25 during exocytosis of histamine by rat gastric enterochromaffin-like cells*. Endocrinology, 1997. **138:**5518-5526.
4. Zanner, R., M. Gratzl and C. Prinz, *Expression of the endocytic proteins dynamin and amphiphysin in rat gastric enterochromaffin-like cells.* J Cell Sci, 2004 **117:**2369-2376.
5. Erspamer, V. *Occurenceof indolealkylamines in nature. In Handbuch der Experimentellen Pharmakologie,* 1966. Eichler, O. & A. Farah, Eds. 132-181. Springer. Berlin.
6. Cetin,Y., et al., *Enterochromaffin cells of the digestive system: cellular source of guanylin, a guanylate cyclase-activating peptide.* Proc Natl Acad Sci USA, 1994. **91:**2935-2939.
7. Peter, D., et al., *Differential expression of two vesicular monoamine transporters.* J. Neurosci, 1995. **15:**6179-6188.
8. Portela-Gomes, G.M., L. Grimelius and R. Bergstrom, *Enterochromaffin (Argentaffin) cells of the rat gastrointestinal tract. An ultrastructural study. Acta Pathol.* Microbiol Immunol Scand [A], 1984. **92:**83-89.
9. Kellum, J.M., et al., *Stroking human jejunal mucosa induces 5-HT release and Cl- secretion via afferent neurons and 5-HT4 receptors*. Am J Physiol Gastrointest Liver Physiol, 1999. **277:**G515-G520.
10. Yu, P.L., et al., *Mechanisms in regulating the release of serotonin from the perfused rat stomach.* Am J Physiol Gastrointest Liver Physiol, 2001. **280:**G1099-G1105.
11. Gershon, M.D. *Review article: roles played by 5-hydroxytryptamine in the physiology of the bowel Aliment*. Pharmacol. Ther, 1999. **13 Suppl 2:**15-30.
12. Hardcastle, J., P.T. Hardcastle and J.S. Redfern, *Action of 5-hydroxytryptamine on intestinal ion transport in the rat*. J Physiol, 1981. **320:**41-55.
13. Sidhu, M., and H.J. Cooke, *Role for 5-HT and ACh in submucosal reflexes mediating colonic secretion.* Am J Physiol Gastrointest Liver Physiol, 1995. **269:**G346-G351.
14. Cooke, H.J., M. Sidhu and Y.Z. Wang, *Activation of 5-HT1P receptors on submucosal afferents subsequently triggers VIP neurons and chloride secretion in the guinea-pig colon*. J Auton Nerv Syst, 1997. **66:**105-110.
15. Peterson, J.W., et al., *Molecular mediators formed in the small intestine in response to cholera toxin*. J Diarrhoeal Dis Res, 1993. **11:**227-234.
16. Hansen, M.B. and E. Skadhauge, *New aspects of the pathophysiology and treatment of secretory diarrhoea.* Physiol. Res, 1995. **44:**61-78.
17. Viner, C.V., et al., *Ondansetron--a new safe and effective antiemetic in patients receiving high-dose melphalan.* Cancer Chemother Pharmacol, 1990. **25:**449-453.
18. Schwörer, H., K. Racke and H. Kilbinger, *Cisplatin increases the release of 5-hydroxytryptamine (5-HT) from the isolated vascularly perfused small intestine of the guinea-pig: involvement of 5-HT3 receptors.* Naunyn Schmiedebergs Arch Pharmacol, 1991. **344:**143-149.

19. Talley, N.J. *Drug therapy options for patients with irritable bowel syndrome.* Am J Manag Care, 2001. **7:**S261-S267.
20. Camilleri, M. *Management of the irritable bowel syndrome* Gastroenterology, 2001. **120:**652-668.
21. Schwörer, H., K. Racke and H. Kilbinger, *Cholinergic modulation of the release of 5-hydroxytryptamine from the guinea pig ileum*. Naunyn Schmiedebergs Arch. Pharmacol, 1987. **336:**127-132.
22. Schinkel, A.H., et al., *P-glycoprotein in the blood-brain barrier of mice influences the brain penetration and pharmacological activity of many drugs* J Clin Invest, 1996. **97:**2517-2524.
23. Lomax, R.B., et al., *L-Type calcium channels in enterochromaffin cells from guinea pig and human duodenal crypts: an in situ study*. Gastroenterology, 1999. **117:**1363-1369.
24. Prinz, C., M. et al., *Histamine secretion from rat enterochromaffinlike cells,* Gastroenterology, 1993. **105:**449-461.
25. Podolsky, D.K. *Regulation of intestinal epithelial proliferation: a few answers, many questions*. Am J Physiol Gastrointest Liver Physiol, 1993. **264:**G179-G186.
26. Racke, K. and H. Schwörer, *Nicotinic and muscarinic modulation of 5-hydroxytryptamine (5-HT) release from porcine and canine small intestine.* Clin Investig, 1992. **70:**190-200.
27. Racke, K., et al., *Evidence that neuronally released vasoactive intestinal polypeptide inhibits the release of serotonin from enterochromaffin cells of the guinea pig small intestine*. Acta Endocrinol (Copenh), 1991. **124:**203-207.
28. Racke, K. and H. Schwörer, *Regulation of serotonin release from the intestinal mucosa*. Pharmacol Res, 1991. **23:**13-25.
29. Racke, K., et al., *Regulation of 5-HT release from enterochromaffin cells*. Behav Brain Res, 1996. **73:**83-87.
30. El Mestikawy, S., et al., *Characterization of an atypical member of the $Na^+/Cl^-$-dependent transporter family: chromosomal localization and distribution in GABAergic and glutamatergic neurons in the rat brain.* J Neurochem, 1994. **62:**445-455.
31. Chen, J.J., et al., *Maintenance of serotonin in the intestinal mucosa and ganglia of mice that lack the high-affinity serotonin transporter: Abnormal intestinal motility and the expression of cation transporters.* J Neurosci, 2001. **21:**6348-6361.
32. Blein, S., E. Hawrot and P.Barlow, *The metabotropic GABA receptor: molecular insights and their functional consequences* Cell Mol. Life Sci, 200. **57:** 635-650.
33. Nakajima, K., et al., *Immunohistochemical demonstration of GABAB receptors in the rat gastrointestinal tract.* Neurochem Res, 1996. **21:**211-215.
34. Cull-Candy, S., S. Brickley and M. Farrant, *NMDA receptor subunits: diversity, development and disease*. Curr Opin Neurobiol, 2001. **11:**327-335.
35. Crasto, C., M.S. Singer and G.M. Shepherd, *The olfactory receptor family album*. Genome Biol, 2001. **2:**1027-1037.

36. Sánchez-Franco, F., et al., *Arginine-vasopressin immunoreactive material in the gastrointestinal tract.* Histochemistry, 1986. **85:**419-422.
37. Chiu, T., et al., *Vasopressin-mediated mitogenic signaling in intestinal epithelial cells.* Am J Physiol Cell Physiol, 2002. **282:**C434-C450.
38. Schurmann, G., et al., *Secretoneurin: a new peptide in the human enteric nervous system.* Histochem. Cell Biol, 1995. **104:**11-19.