ELSEVIER

European Journal of Pharmacology 387 (2000) 19–25

ejp

www.elsevier.nl/locate/ejphar

Histamine depletion does not affect pancreastatin secretion from isolated rat stomach ECL cells

Erik Lindström, Per Norlén, Rolf Håkanson *

Institute of Physiological Sciences, Department of Pharmacology, University of Lund, Sölvegatan 10, S-223 62 Lund, Sweden

Received 14 June 1999; received in revised form 7 October 1999; accepted 15 October 1999

Abstract

ECL cells co-secrete histamine and pancreastatin, a chromogranin A-derived peptide, in response to gastrin. The aim of the study was to explore possible ways to deplete ECL cells of histamine without affecting pancreastatin and to examine how histamine depletion affects pancreastatin secretion. Isolated rat stomach ECL cells (80–85% purity), prepared by counter-flow elutriation, were cultured for 48 h in the presence of α-fluoromethylhistidine (histidine decarboxylase inhibitor), bafilomycin A_1 (inhibitor of vacuolar-type proton-translocating ATPase) or reserpine (inhibitor of vesicular monoamine transporter). At this stage, the cells were challenged with 10 nM (EC_{100}) gastrin-17 for 30 min. Histamine and pancreastatin were determined by radioimmunoassay. Maximally effective concentrations of α-fluoromethylhistidine, bafilomycin A_1 and reserpine were found to lower ECL-cell histamine (by 60%, 78% and 80%, respectively) without affecting pancreastatin. Basal histamine secretion was reduced in a dose-dependent manner by all three drugs. Gastrin-evoked histamine secretion was reduced greatly by the three agents, while pancreastatin secretion was unaffected. The results show that histamine can be depleted not only by inhibiting its formation (α-fluoromethylhistidine), but also (and more effectively) by inhibiting histamine vesicular uptake, directly (reserpine) or indirectly (bafilomycin A_1). The results also indicate that although histamine is co-stored with pancreastatin, it is not required for either storage or secretion of pancreastatin.

Keywords: Bafilomycin A_1; α-Fluoromethylhistidine; Histidine decarboxylase; Reserpine; Vacuolar-type ATPase; Vesicular monoamine transporter

1. Introduction

The ECL cell is the predominant endocrine/paracrine cell type in the oxyntic mucosa of the rat stomach (Håkanson et al., 1994). The paracrine role of the ECL cell is to secrete histamine, which in turn stimulates parietal cells to secrete hydrochloric acid (Håkanson et al., 1986; Sandvik et al., 1987; Waldum et al., 1991; Andersson et al., 1996). Besides histamine, the ECL cells produce, store and secrete chromogranin A-derived peptides, such as pancreastatin (Chen et al., 1994; Håkanson et al., 1995). Both histamine and the chromogranin A-derived peptides are thought to be stored together in secretory vesicles (Chen et al., 1994, 1996a; Lindström et al., 1997; Zhao et al., 1999a), although in different compartments within the vesicle (Zhao et al., 1999a). Indeed, gastrin stimulation results in the parallel secretion of histamine and pancreastatin, as demonstrated in studies using isolated perfused rat stomachs (Chen et al., 1996b) and isolated rat stomach ECL cells (Lindström et al., 1997).

Histamine is formed from histidine by the cytosolic enzyme histidine decarboxylase. Administration of α-fluoromethylhistidine, an irreversible inhibitor of histidine decarboxylase (Kollonitsch et al., 1978), reduces oxyntic mucosal histamine by about 80% after 48 h (Andersson et al., 1992). α-Fluoromethylhistidine is thus a valuable tool in exploring the significance of ECL-cell histamine (Andersson et al., 1996).

Amine uptake into secretory vesicles in neurons and endocrine cells is mediated by a vesicular monoamine transporter (for a review see Schuldiner et al., 1995). Two vesicular monoamine transporter isoforms have been characterized: type 1 and type 2 (Erickson et al., 1992; Liu et al., 1992). They differ in structure and substrate affinity, the apparent affinity of histamine being 10–100-fold higher for vesicular monoamine transporter type 2 than for vesicular monoamine transporter type 1 (Peter et al., 1994, 1996; Merickel and Edwards, 1995). Indeed, vesicular

* Corresponding author. Tel.: +46-46-222-75-85; fax: +46-46-222-44-29.

E-mail address: rolf.hakanson@farm.lu.se (R. Håkanson)

0014-2999/00/$ - see front matter
PII: S0014-2999(99)00726-8

monoamine transporter type 2 has been demonstrated in ECL cells of the rat oxyntic mucosa (Weihe et al., 1994; Erickson et al., 1995; Peter et al., 1995; De Giorgio et al., 1996; Dimaline and Struthers, 1996; Zhao et al., 1997).

Reserpine binds to the amine recognition site of vesicular monoamine transporter type 2, preventing amine transport into storage granules/vesicles, thereby causing a gradual depletion of amine (Kirshner, 1962; Slotkin and Edwards, 1973; Håkanson et al., 1974; Scherman and Henry, 1984). The effect of reserpine is irreversible in the sense that the effect does not subside until new storage granules/vesicles have replaced those that have been inactivated (Stitzel, 1977; Rudnick et al., 1990).

Vesicular monoamine transporters utilize a H^+ gradient to drive the accumulation of amines into the storage granules/vesicles (as reviewed by Njus et al., 1986; Johnson, 1988). The proton gradient is dependent upon vacuolar-type ATPases which are present in all neurotransmitter storage vesicles studied so far (Schuldiner et al., 1995). Vacuolar-type ATPases can be inhibited by bafilomycins (Bowman et al., 1988). Indeed, vacuolar-type ATPase inhibition was found to inhibit acidification of granules in isolated ECL cells (Prinz et al., 1993, 1994). However, the effects of the selective vacuolar-type ATPase inhibitor bafilomycin A_1 on ECL-cell histamine uptake and storage have not been studied.

Previous observations have indicated that ECL-cell histamine and pancreastatin are secreted in parallel in response to gastrin or pituitary adenylate cyclase activating peptide (Lindström et al., 1997). Also, inhibitory agents such as somatostatin, galanin and prostaglandins E_1 and E_2 inhibit the secretion of both histamine and pancreastatin (Prinz et al., 1993, 1997; Lindström and Håkanson, 1998; Lindström et al., 1997). Although pancreastatin and histamine are thought to be co-stored in the secretory vesicles (Lindström et al., 1997; Zhao et al., 1999a), which are numerous in the cytoplasm of the ECL cells, histamine does not seem to be necessary for pancreastatin secretion to occur (Chen et al., 1996b). The aim of the present study was to examine the effects of α-fluoromethylhistidine, bafilomycin A_1 and reserpine on ECL-cell stores of histamine and pancreastatin and on basal and gastrin-stimulated secretion of these two secretory products.

2. Materials and methods

2.1. Chemicals

Reserpine was from Sigma (St. Louis, MO, USA). A stock solution (0.01 M) was prepared by dissolving reserpine in 100% dimethylsulfoxide. Bafilomycin A_1 was a

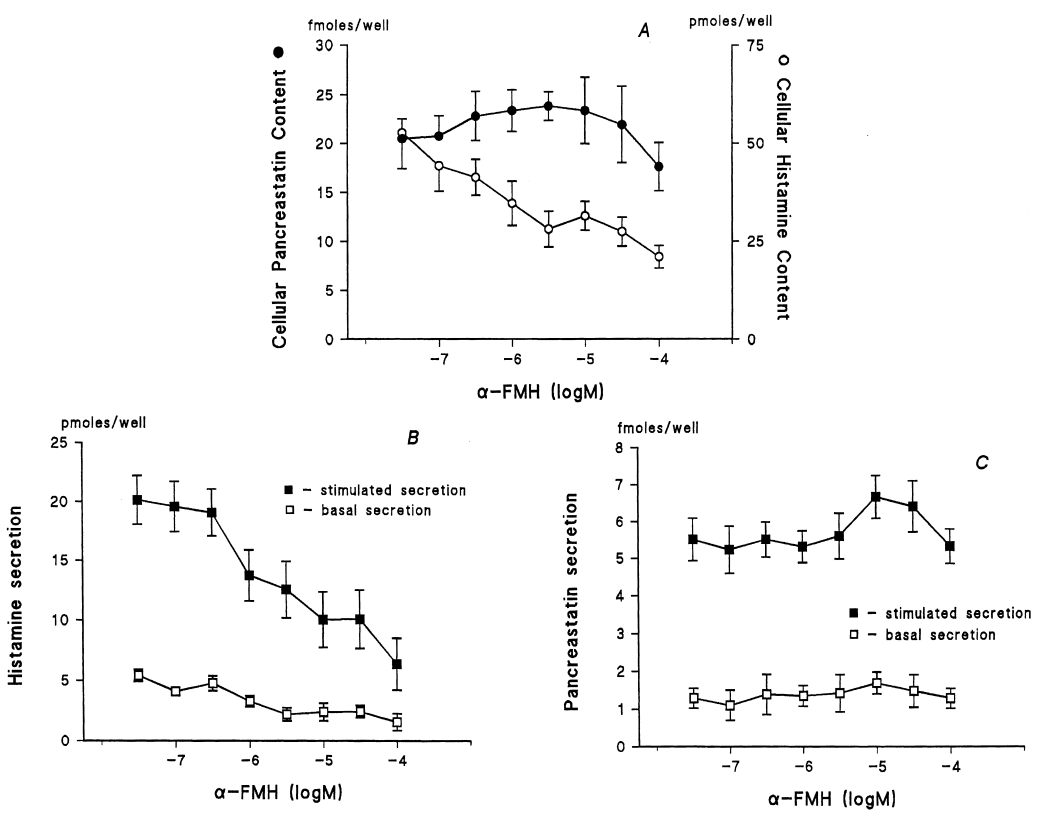

Fig. 1. Effect of α-fluoromethylhistidine on isolated ECL cells. Treatment with various concentrations of α-fluoromethylhistidine for 48 h lowers ECL-cell histamine (○) without affecting pancreastatin (●) (A) Pretreatment with α-fluoromethylhistidine for 48 h also reduces basal and gastrin-stimulated (10 nM) histamine secretion (B) without affecting pancreastatin secretion (C). Means ± SEM, $n = 6–8$.

gift from Dr. Birgitta Ryberg (Astra Hässle, Mölndal, Sweden). A stock solution (0.01 M) was prepared by dissolving the drug in ethanol. α-Fluoromethylhistidine was from Research Biochemical International (Natick, MA, USA). A 0.1 M stock solution was prepared by dissolving the drug in 0.9% NaCl. Rat gastrin-17 was from Research Plus (Bayonne, NJ, USA). Anti-rat histidine decarboxylase antiserum, raised in a guinea pig, was a gift from Dr. Lo Persson, Lund, Sweden (Dartsch and Persson, 1998). Tetramethylrhodamine isothiocyanate (TRITC)-conjugated donkey anti-guinea pig immunoglobulin G (IgG) was from Jackson ImmunoResearch (West Grove, PA, USA). Matrigel® was from Collaborative Biomedical Products (Bedford, MA, USA). Dulbecco's Modified Eagle's Medium (DMEM)–Ham's F12 was from Sigma (St. Louis).

2.2. Isolation, fractionation and primary culture of ECL cells

The ECL cells were purified as described in detail previously (Lindström et al., 1997). Briefly, cells were harvested from the oxyntic mucosa of four rats at a time, and repeated cell elutriation resulted in an average yield of 2 million cells. The purity of the ECL cell preparation was assessed by immunocytochemistry, using a guinea pig anti-rat histidine decarboxylase antiserum (1:750) and found to be 80–85%. A drop of the cell suspension was applied to glass slides and smears were prepared. After fixation in 4% neutral formaldehyde, a drop of primary antiserum was placed on each smear. The reaction took place in a humid chamber at 4°C overnight. Immunoreaction was visualized by means of TRITC-conjugated donkey anti-guinea pig IgG (1:80).

The cells were cultured in 96 well plates pre-coated with Matrigel® (diluted 1:10 with DMEM–Ham's F12) (20,000 cells per well) in a humid atmosphere with 5% CO_2/95% air at 37°C for 48 h until the start of the experiments. The culture medium consisted of DMEM–Ham's F12 (1:1) supplemented with 2% fetal calf serum, 2 mM glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin, 250 ng/ml amphotericin B, 10 mg/ml insulin, 5.5 mg/ml transferrin, 5 μg/ml selenium, 0.5 mg/ml bovine serum albumin, 10 nM hydrocortisone, 15 mM HEPES, 10 μM pyridoxal-5-phosphate and 100 pM gastrin-17. α-Fluoromethylhistidine (final concentration 0.1 μM–0.1 mM), bafilomycin A_1 (final concentration 0.1 pM–10 nM) or reserpine (final concentration 0.1 pM–0.1 μM) were added to the medium at the start of culture.

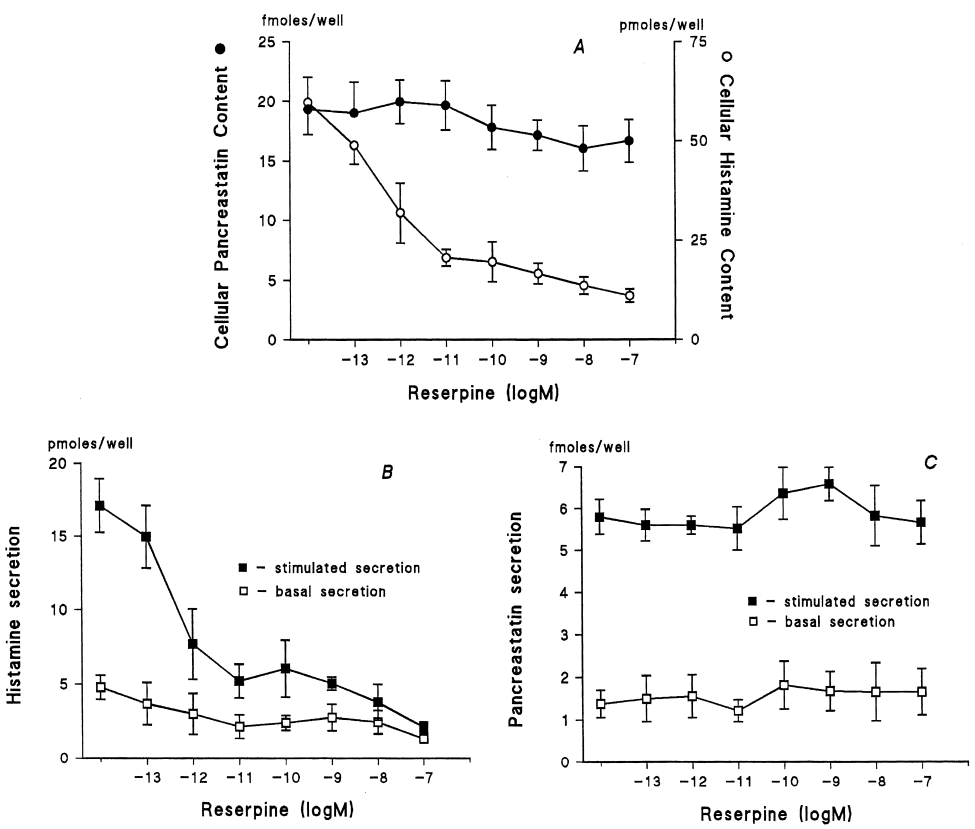

Fig. 2. Effect of reserpine on isolated ECL cells. Treatment with various concentrations of reserpine for 48 h depletes ECL-cell histamine (○) without affecting pancreastatin (●) (A) Pretreatment with reserpine for 48 h also reduces gastrin-stimulated (10 nM) histamine secretion (B) without affecting pancreastatin secretion (C). Means ± SEM, $n = 6–8$.

2.3. Secretion studies

ECL cells in culture were exposed to various concentrations of α-fluoromethylhistidine, bafilomycin A_1 or reserpine for 48 h. At that stage, the cells were either collected for measurement of histamine/pancreastatin content or challenged with gastrin in studies of histamine/pancreastatin secretion. For the measurement of histamine/pancreastatin content, the cells were exposed to boiling redistilled water for 2×5 min. The aqueous extracts were collected and stored at −20°C until radioimmunoassay of histamine and pancreastatin. For the study of secretion, the culture medium was replaced with fresh serum-free culture medium without gastrin but containing α-fluoromethylhistidine, bafilomycin A_1 or reserpine. After equilibration for 2–3 h, the medium was again aspirated and replaced with secretion medium (mM): 150 NaCl, 5 KCl, 2 $CaCl_2$, 10 HEPES at pH 7.0, without α-fluoromethylhistidine, bafilomycin A_1 or reserpine. Basal and stimulated (10 nM gastrin) secretion was measured (30-min incubation). The incubation was interrupted by centrifuging the plates at $220 \times g$ for 1 min. The supernatants were collected and stored at −20°C until measurement of pancreastatin and histamine.

2.4. Determination of pancreastatin

The pancreastatin-like immunoreactivity was measured by radioimmunoassay using authentic rat pancreastatin as standard (Håkanson et al., 1995). The amount of pancreastatin released was expressed as fmol equivalents of rat pancreastatin per well (100 μl).

2.5. Determination of histamine

Histamine was measured using a commercial radio-immunoassay kit (Immunotech, Marseille, France) with a detection limit of 0.2 nM histamine. The amount of histamine released into each well (20,000 cells) was expressed as pmol/well (100 μl).

2.6. Statistical analysis

All results are presented as mean ± SEM. n is the number of cell preparations. The effect of the various drugs on basal histamine secretion was analyzed by one-way analysis of variance (ANOVA) followed by linear trend analysis. $P < 0.05$ was considered significant. IC_{50} concentrations (i.e., the concentration evoking a 50% re-

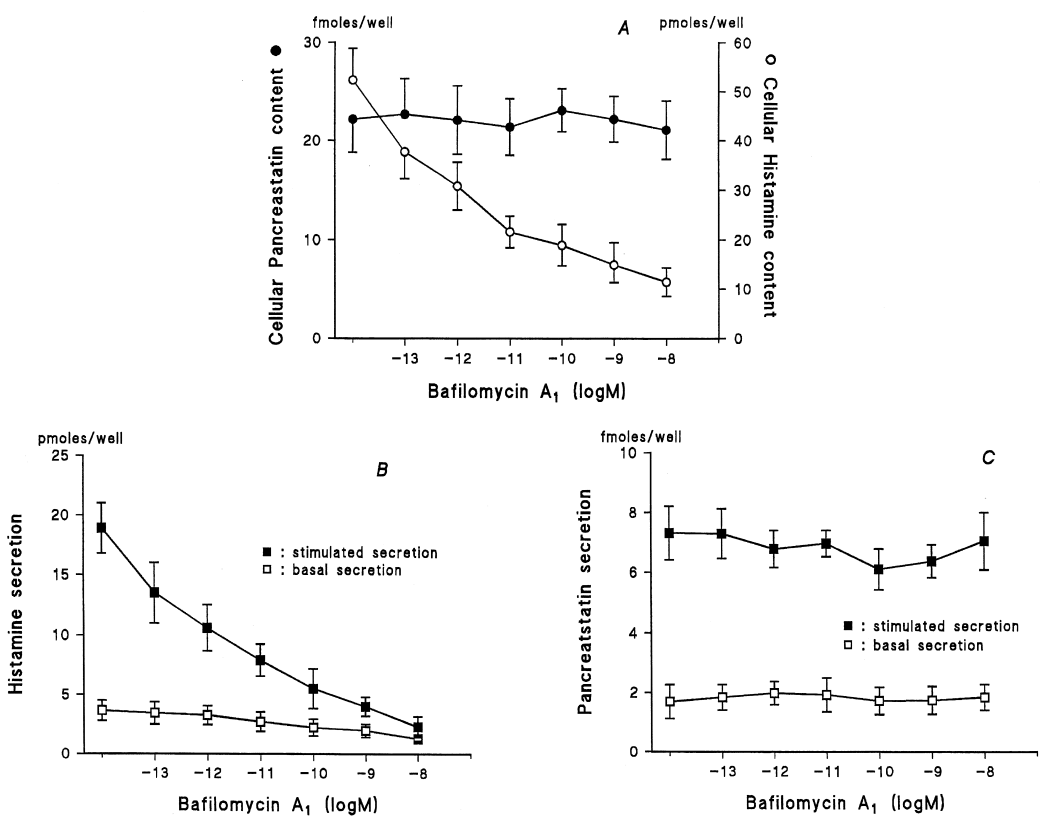

Fig. 3. Effect of bafilomycin A_1 on isolated ECL cells. Treatment with various concentrations of bafilomycin A_1 for 48 h depletes ECL-cell histamine (○) without affecting pancreastatin (●) (A) Pretreatment with various concentrations of bafilomycin A_1 for 48 h also reduces gastrin-stimulated (10 nM) histamine secretion (B) without affecting pancreastatin secretion (C). Means ± SEM, $n = 6–8$.

duction of the maximal inhibitory effect) was calculated using the GRAPH PAD PRISM software Graphied Software Inc., San Diego, CA, USA).

3. Results

3.1. Effect of α-fluoromethylhistidine

α-Fluoromethylhistidine treatment for 48 h reduced the histamine content in a concentration-dependent manner (Fig. 1A). A near-maximally effective concentration of α-fluoromethylhistidine (100 μM) caused a 60% drop in ECL-cell histamine. The IC_{50} value was 0.7 ± 0.3 μM. The pancreastatin stores were unaffected (Fig. 1A). Gastrin (10 nM) induced a 3.7-fold increase in histamine secretion and and a 4.3-fold increase in pancreastatin secretion in control cells (Fig. 1B). Cells pre-treated with α-fluoromethylhistidine displayed a reduced histamine response to gastrin (Fig. 1B). The effect of α-fluoromethylhistidine was concentration-dependent and the IC_{50} was 1.2 ± 0.5 μM. Basal histamine secretion was reduced significantly (29% remaining, $P < 0.001$). Neither basal nor gastrin-stimulated pancreastatin secretion was affected (Fig. 1C).

3.2. Effect of reserpine

Treatment with reserpine for 48 h depleted ECL-cell histamine in a concentration-dependent manner (Fig. 2A). The IC_{50} value was 1.5 ± 0.5 pM. A near-maximal concentration of reserpine (100 nM for 48 h) caused an 80% drop in the histamine content. By contrast, the ECL-cell pancreastatin content was unaffected (Fig. 2A). Gastrin (10 nM) induced a 3.6-fold increase in histamine secretion and a 4.2-fold increase in pancreastatin secretion in control cells (Fig. 2B). Reserpine greatly reduced the ability of the ECL cells to secrete histamine in response to gastrin (Fig. 2B). The IC_{50} value was 0.3 ± 0.1 pM. Basal histamine secretion was reduced by 72% ($P < 0.05$). Neither basal nor stimulated pancreastatin secretion was affected by reserpine treatment (Fig. 2C).

3.3. Effect of bafilomycin A_1

Treatment with bafilomycin A_1 for 48 h lowered ECL-cell histamine but not pancreastatin (Fig. 3A). A maximally effective concentration of bafilomycin A_1 (10 nM) induced a 78% drop in histamine (IC_{50} value: 3.3 ± 1.0 pM) without affecting pancreastatin (higher concentrations of bafilomycin A_1 could not be tested due to cell death). ECL cells pre-treated with bafilomycin A_1 displayed a much reduced ability to secrete histamine in response to gastrin (IC_{50} value: 2.6 ± 1.1 pM) (Fig. 3B), while pancreastatin secretion was unaffected (Fig. 3C). Basal histamine secretion was reduced by 65% ($P < 0.01$).

4. Discussion

Despite their different origins, histamine and pancreastatin-like immunoreactivity are thought to be co-stored in the secretory vesicles of the ECL cells and co-secreted from these cells (Lindström et al., 1997; Zhao et al., 1999a). However, histamine and pancreastatin occur in different compartments within the secretory organelle (see Fig. 4). The pancreastatin precursor chromogranin A is deposited in the secretory organelles as they are assembled in the Golgi apparatus, whereas histamine is produced in the cytosol and continuously taken up from the cytosol by the granules/secretory vesicles through the action of vesicular monoamine transporter type 2 (Zhao et al., 1999a), which accumulates one molecule of histamine in exchange for two protons (Schuldiner et al., 1995). Thus, the histamine content of the secretory organelles can be lowered by various pharmacological manipulations (Fig. 4) that do not affect the pancreastatin content. In vivo, α-fluoromethylhistidine inhibits the histamine-forming enzyme irreversibly, thereby inducing loss of histamine but not of pancreastatin (Chen et al., 1996b), and the vesicular monoamine transporter type 2 inhibitor reserpine prevents accumulation of histamine in the secretory organelles without affecting pancreastatin (Zhao et al., 1999b). Finally, bafilomycin A_1 is an inhibitor of the proton-translocating enzyme (vacuolar-type ATPase) (Bowman et al., 1988),

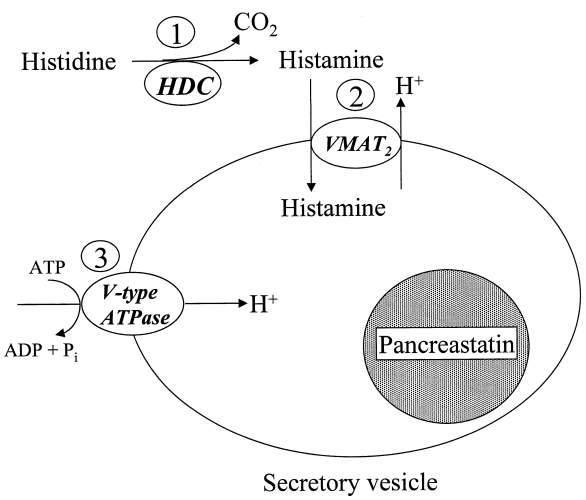

Fig. 4. Intracellular sites of action for α-fluoromethylhistidine, reserpine and bafilomycin A_1: α-fluoromethylhistidine (irreversible inhibition of cytosolic histidine decarboxylase (HDC), 1), reserpine (irreversible blockade of vesicular monoamine transporter type 2 ($VMAT_2$) in the secretory vesicle membrane, 2) and bafilomycin A_1 (blockade of vacuolar-type ATPase (V-type ATPase) in the secretory vesicular membrane, 3). The documented effects of bafilomycin A_1 and reserpine are in line with the view that a proton drive coupled to vesicular monoamine transporter type 2 is necessary for the accumulation of histamine in the secretory vesicles. Pancreastatin is stored in the dense core of the secretory vesicles along with other chromogranin A-derived peptides and proteins. Histamine is probably in the electron–lucent part of the secretory vesicles (Zhao et al., 1999b).

which is responsible for creating the proton drive that makes vesicular amine uptake possible. Hence, it can be expected to influence ECL-cell histamine stores indirectly by preventing vesicular monoamine transporter type 2 from operating properly.

Long-term treatment (48 h) of isolated ECL cells with α-fluoromethylhistidine, reserpine or bafilomycin A_1 caused a marked depletion of histamine. As a consequence, basal and stimulated secretion of histamine was reduced. In contrast, storage and secretion of pancreastatin were unaffected. While α-fluoromethylhistidine and bafilomycin A_1 were equally potent in depleting histamine and in blocking histamine secretion, reserpine was found to be more potent in blocking histamine secretion than in depleting histamine (IC_{50}: 0.3 vs. 1.5 pM). The reason for this is unknown. The fact that treatment with α-fluoromethylhistidine, reserpine or bafilomycin A_1 failed to affect gastrin-evoked pancreastatin secretion indicates that ''normal'' histamine levels in ECL cells are not required for pancreastatin secretion to occur.

α-Fluoromethylhistidine, which blocks histamine formation, lowered the ECL-cell histamine content quite effectively. However, reserpine and bafilomycin A_1, which interfere with accumulation and storage of histamine, depleted ECL-cell histamine to an even greater extent. This is surprising if we assume that histamine formation goes on undisturbed, resulting in a continuous supply of newly formed histamine. If storage in granules/vesicles is prevented, histamine can be expected to accumulate in the cytosol. Such accumulation does not seem to occur, however. One possible explanation is that cytosolic histamine, prevented by reserpine or bafilomycin A_1 from accumulating in the secretory granules/vesicles, leaks out from the cell. Another possibility is that cytosolic histamine is promptly degraded. There was no evidence that basal release (leakage) of ECL-cell histamine into the medium was enhanced by either reserpine of bafilomycin A_1. Consequently, histamine leakage, if it occurs, must be very slow in order to be virtually imperceptible. Histamine degradation occurs either by *N*-methylation of the imidazole ring (catalyzed by histamine *N*-methyltransferase) or by oxidative deamination (catalyzed by diamine oxidase). Whether these enzymes occur in the ECL cells and whether they play a role in the degradation of cytosolic ECL-cell histamine is unknown. Adding inhibitors of *N*-methyl histamine transferase (10 μM metoprin) (Duch et al., 1978) and diamine oxidase (1 μM aminoguanidine) (Kahlson et al., 1963) did not prevent the reserpine-induced depletion of ECL-cell histamine (Lindström, unpublished observations). Conceivably, elevated cytosolic histamine suppresses the histidine decarboxylase activity. This will be the subject of a separate study.

We conclude that despite the fact that histamine and pancreastatin are stored together in the same population of secretory vesicles, pancreastatin is stored and secreted independently of histamine. In addition, the results are in line with the view that a proton-translocating vacuolar-type ATPase causes protons to accumulate in the secretory vesicles of the ECL cell. Vesicular monoamine transporter type 2 in the vesicle membrane enables the secretory vesicles to exchange protons for histamine, thereby controlling the pH of the interior. The resulting pH will reflect the proton/histamine ratio and will depend on the combined activities of vacuolar-type ATPase, vesicular monoamine transporter type 2 and histidine decarboxylase. It is probable that the pH of the interior of the secretory vesicles will have consequences for the rate by which secretory proteins/peptides are being processed, as has been shown in other endocrine cells (Wolkersdorfer et al., 1996; Voronina et al., 1997).

Amines are costored with chromogranin A-derived peptides and peptide hormones in many endocrine cells. The histamine-storing ECL cells may perhaps be viewed as representative of this category of cells. If so, it can be concluded that amines are not a prerequisite for the ability of such cells to store and secrete peptide hormones.

References

Andersson, K., Chen, D., Håkanson, R., Mattsson, H., Sundler, F., 1992. Enterochromaffin-like cells in the rat stomach: effect of α-fluoromethylhistidine-evoked histamine depletion. A chemical, histochemical and electronmicroscopic study. Cell Tissue Res. 270, 7–13.

Andersson, K., Cabero, J.L., Mattsson, H., Håkanson, R., 1996. Gastric acid secretion after depletion of enterochromaffin-like cell histamine. Scand. J. Gastroenterol. 31, 24–30.

Bowman, E.J., Siebers, A., Altendorf, K., 1988. Bafilomycins: a class of inhibitors of membrane ATPases from microorganisms, animal cells and plant cells. Proc. Natl. Acad. Sci. U.S.A. 85, 7972–7976.

Chen, D., Monstein, H.-J., Nylander, A.-G., Zhao, C.-M., Sundler, F., Håkanson, R., 1994. Acute responses of rat stomach enterochromaffinlike cells to gastrin. Secretory activation and adaption. Gastroenterology 107, 18–27.

Chen, D., Zhao, C.-M., Andersson, K., Sundler, F., Håkanson, R., 1996a. Ultrastructure of enterochromaffin-like cells in rat stomach: effects of α-fluoromethylhistidine-evoked histamine depletion and hypergastrinemia. Cell Tissue Res. 283, 469–478.

Chen, D., Mårvik, R., Rønning, K., Andersson, K., Waldum, H.L., Håkanson, R., 1996b. Gastrin-evoked secretion of pancreastatin and histamine from ECL cells and of acid from parietal cells in isolated, vascularly perfused rat stomach. Effects of isobutyl methylxanthin and α-fluoromethylhistidine. Regul. Pept. 65, 133–138.

Dartsch, C., Persson, L., 1998. Recombinant expression of rat histidine decarboxylase: generation of antibodies useful for western blot analysis. Int. J. Biochem. Cell Biol. 30, 773–782.

De Giorgio, R., Su, D., Peter, D., Edwards, R.H., Brecha, N.C., Sternini, C., 1996. Vesicular monoamine transporter 2 expression in enteric neurons and enterochromaffin-like cells of the rat. Neurosci. Lett. 217, 77–80.

Dimaline, R., Struthers, J., 1996. Expression and regulation of a vesicular monoamine transporter in rat stomach: a putative histamine transporter. J. Physiol. (London) 490, 249–256.

Duch, S.D., Bowers, S.W., Nichol, C.A., 1978. Elevation of brain histamine levels by diaminopyrimidine inhibitors of histamine *N*-methyltransferase. Biochem. Pharmacol. 27, 1507–1509.

Erickson, J.D., Eiden, L.E., Hoffman, B.J., 1992. Expression cloning of a reserpine-sensitive vesicular monoamine transporter. Proc. Natl. Acad. Sci. U.S.A. 89, 10993–10997.

Erickson, J.D., Eiden, L.E., Schäfer, M.K.-H., Weihe, E., 1995. Reserpine- and tetrabenazine-sensitive transport of ^{3}H-histamine by the neuronal isoform of the vesicular monoamine transporter. J. Mol. Neurosci. 6, 277–287.

Håkanson, R., Liedberg, G., Rehfeld, J.F., Sundler, F., Ericson, L.E., 1974. Effect of reserpine on serum gastrin concentration and on mucosal amines and amino acid decarboxylase activities in rat stomach. J. Pharmacol. Exp. Ther. 189, 603–615.

Håkanson, R., Böttcher, G., Ekblad, E., Panula, P., Simonsson, M., Dohlsten, M., Hallberg, T., Sundler, F., 1986. Histamine in endocrine cells in the stomach: a survey of several species using a panel of histamine antibodies. Histochemistry 86, 5–17.

Håkanson, R., Chen, D., Sundler, F., 1994. The ECL cells. In: Johnson, L.R. (Ed.), Physiology of the Gastrointestinal Tract 2 Raven Press 3rd edn., New York, pp. 1171–1184.

Håkanson, R., Ding, X.-Q., Norlén, P., Chen, D., 1995. Circulating pancreastatin is a marker for the enterochromaffin-like cells of the rat stomach. Gastroenterology 108, 1445–1452.

Johnson, R., 1988. Accumulation of biological amines in chromaffin granules: a model for hormone and neurotransmitter transport. Physiol. Rev. 68, 232–307.

Kahlson, G., Rosengren, E., Thunberg, R., 1963. Observations on the inhibition of histamine formation. J. Physiol. (London) 169, 467–486.

Kirshner, N., 1962. Uptake of catecholamines by a particulate fraction of the bovine medulla. J. Biol. Chem. 237, 2311–2317.

Kollonitsch, J., Perkins, L.M., Patchett, A.A., Doldouras, G.A., Marburg, S., Duggan, D.E., Maycock, A.L., Aster, S.D., 1978. Selective inhibitors of biosynthesis of aminergic neurotransmitters. Nature 274, 906–908.

Lindström, E., Håkanson, R., 1998. Prostaglandins inhibit secretion of histamine and pancreastatin from isolated rat stomach ECL cells. Br. J. Pharmacol. 124, 1307–1313.

Lindström, E., Björkqvist, M., Boketoft, Å., Chen, D., Zhao, C.-M., Kimura, K., Håkanson, R., 1997. Neurohormonal regulation of histamine and pancreastatin secretion from isolated rat stomach ECL cells. Regul. Pept. 71, 73–86.

Liu, Y., Peter, D., Roghani, A., Schuldiner, S., Prive, G., Eisenberg, D., Brecha, N., Edwards, R.H., 1992. A cDNA that suppresses MPP^{+} toxicity encodes a vesicular amine transporter. Cell 70, 539–551.

Merickel, A., Edwards, R.H., 1995. Transport of histamine by vesicular monoamine transporter-2. Neuropharmacology 34, 1543–1547.

Njus, D., Kelley, P.M., Harnadek, G.J., 1986. Bioenergetics of secretory vesicles. Biochim. Biophys. Acta 853, 237–265.

Peter, D., Jiminez, J., Liu, Y., Kim, J., Edwards, R., 1994. The chromaffin granule and synaptic vesicle transporters differ in substrate recognition and sensitivity to inhibitors. J. Biol. Chem. 269, 7231–7237.

Peter, D., Liu, Y., Sternini, C., de Giorgio, R., Brecha, N., Edwards, R.H., 1995. Differential expression of two vesicular monoamine transporters. J. Neurosci. 15, 6179–6188.

Peter, D., Vu, T., Edwards, R.H., 1996. Chimeric vesicular monoamine transporters identify structural domains that influence substrate affinity and sensitivity to tetrabenazine. J. Biol. Chem. 271, 2979–2986.

Prinz, C., Kajimura, M., Scott, D.R., Mercier, F., Helander, H., Sachs, G., 1993. Histamine secretion from rat enterochromaffinlike cells. Gastroenterology 105, 449–461.

Prinz, C., Scott, D.R., Hurwitz, D., Helander, H., Sachs, G., 1994. Gastrin effects on isolated rat enterochromaffin-like cells in primary culture. Am. J. Physiol. 267, G663–G675.

Prinz, C., Neumayer, N., Mahr, S., Classen, M., Schepp, W., 1997. Functional impairment of rat enterochromaffin-like cells by interleukin 1β. Gastroenterology 112, 364–375.

Rudnick, G., Steiner-Mordoch, S.S., Fishkes, H., Sternbach, Y., Schuldiner, S., 1990. Energetics of reserpine binding and occlusion by the chromaffin granule transporter. Biochemistry 29, 603–608.

Sandvik, A.K., Waldum, H.L., Kleveland, P.M., Schulze Sögnen, B., 1987. Gastrin evokes an immediate and dose-dependent histamine release preceding acid secretion in the totally isolated vascularly perfused rat stomach. Scand. J. Gastroenterol. 22, 803–808.

Scherman, D., Henry, J.-P., 1984. Reserpine binding to bovine chromaffin granule membranes. Mol. Pharmacol. 25, 113–122.

Schuldiner, S., Shirvan, A., Linial, M., 1995. Vesicular neurotransmitter transporters: from bacteria to humans. Physiol. Rev. 75, 369–392.

Slotkin, T.A., Edwards, K., 1973. Effects of reserpine on the content and properties of rat adrenal medullary storage vesicles. Biochem. Pharmacol. 22, 549–560.

Stitzel, R.E., 1977. The biological fate of reserpine. Pharmacol. Rev. 28, 179–205.

Voronina, S., Henry, J., Vaillant, C., Dockray, G.J., Varro, A., 1997. Amine precursor uptake and decarboxylation: significance for processing of the rat gastrin precursor. J. Physiol. (London) 501, 363–374.

Waldum, H.L., Sandvik, A.K., Brenna, E., Petersen, H., 1991. The gastrin–histamine sequence in the regulation of gastric acid secretion. Gut 32, 698–700.

Weihe, E., Schäfer, M.K.-H., Erickson, J.D., Eiden, L.E., 1994. Localization of vesicular monoamine transporter isoforms (VMAT1 and VMAT2) to endocrine cells and neurons in rat. J. Mol. Neurosci. 5, 149–164.

Wolkersdorfer, M., Laslop, A., Lazure, C., Fischer-Colbrie, R., Winkler, H., 1996. Processing of chromogranins in chromaffin cell culture: effects of reserpine and α-methyl-*p*-tyrosine. Biochem. J. 316, 953–958.

Zhao, C.-M., Jacobsson, G., Chen, D., Håkanson, R., Meister, B., 1997. Exocytotic proteins in enterochromaffin-like (ECL) cells of the rat stomach. Cell Tissue Res. 290, 539–551.

Zhao, C.-M., Chen, D., Lintunen, M., Panula, P., Håkanson, R., 1999a. Secretory organelles in ECL cells of the rat stomach. An immunohistochemical and electron-microscopic study. Cell Tissue Res., (in press).

Zhao, C.-M., Chen, D., Lintunen, M., Panula, P., Håkanson, R., 1999b. Effects of reserpine on ECL-cell ultrastructure and histamine compartmentalization in the rat stomach. Cell Tissue Res. 295, 131–140.