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# RESERPINE-INDUCED MOBILIZATION OF HISTAMINE AND VITAMIN $B_{12}$-BINDING PROTEINS FROM A SPECIAL TYPE OF ENDOCRINE CELLS IN RAT STOMACH

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**Abstract**—Reserpine was found to mobilize histamine from a large number of the enterochromaffin-like cells in the oxyntic gland area of the rat stomach, as revealed by fluorescence histochemistry. Chemical estimations demonstrated a 50 per cent reduction in the gastric histamine content upon reserpinization. Histamine persisted in gastric mast cells and in a population of reserpine-resistant enterochromaffin-like cells. The results indicate the existence of three separate pools of gastric histamine.

Vitamin $B_{12}$-binding proteins (including the intrinsic factor) have been suggested to occur in the histamine-containing enterochromaffin-like cells of the rat stomach. Reserpine treatment caused an almost total depletion of gastric vitamin $B_{12}$-binding proteins, whereas the intrinsic factor activity (i.e. the ability to absorb ingested [$^{58}$Co] labelled cyanocobalamin) was only moderately impaired. It is suggested that the vitamin $B_{12}$-binders reside in the reserpine-sensitive population of enterochromaffin-like cells, and that also under conditions of impaired storage, the formation and continuous release of intrinsic factor is sufficient to satisfy the normal demands of vitamin $B_{12}$ absorption.

THE GASTRIC mucosa of the rat contains an extensive system of argyrophil cells[1] which have been classified as endocrine on the basis of certain ultrastructural features, such as a well-developed endoplasmic reticulum, a prominent Golgi apparatus and numerous secretory granules.[2,3] In addition, a considerable portion of the argyrophil cells in the pyloric gland area stain argentaffin, which has been attributed to their 5-hydroxytryptamine (5-HT) content.[4] Fluorescence microscopy has revealed that the argyrophil, non-argentaffin cells of the oxyntic gland area are the major storage site for gastric histamine.[1,5] These endocrine cells are devoid of 5-HT but have the capacity to produce and store a number of arylethylamines after administration of the immediate precursor amino acid, e.g. dopamine after injection of 3,4-dihydroxyphenylalanine (DOPA).[6,7] When silver-stained, the argyrophil, non-argentaffin cells of the oxyntic gland area are morphologically indistinguishable from the 5-HT-containing enterochromaffin (argentaffin) cells in the pyloric gland area. Because of this morphologic similarity, the argyrophil, non-argentaffin cells have been referred to as enterochromaffin-like.[7] There is recent evidence that in the rat, vitamin $B_{12}$-binding proteins, such as the intrinsic factor, are stored within the system of enterochromaffin-like cells.[8]

Reserpine is known to reduce the content of gastric histamine,[9,10] but its cellular site of action is not evident. It may affect the histamine stores of the endocrine cells and/or the gastric mast cells. In previous studies, reserpine has been found to reduce the ability of the enterochromaffin-like cells to accumulate dopamine following the

administration of L-DOPA. However, in the fluorescence microscope a considerable fraction of the enterochromaffin-like cell system appeared unaffected by the reserpine treatment, suggesting the possible existence of two distinct populations of enterochromaffin-like cells, differing in their sensitivity to the amine-depleting action of reserpine. The present report is concerned with the effect of reserpine on the histamine content of the enterochromaffin-like cells and with its possible action on the stores of vitamin $B_{12}$-binding proteins in the gastric mucosa.

## METHODS

The studies were performed on 200–250 g Sprague–Dawley rats of either sex, maintained on a standard pellet diet (SAN-Bolagen, Sweden) and tap water *ad lib.* For the chemical estimations of gastric histamine, dopamine and vitamin $B_{12}$-binding proteins the rats were starved for 24 hr before receiving saline or reserpine and until sacrifice 24 hr later. All animals were killed by decapitation under light ether anesthesia.

*Histochemistry.* Five rats were injected with 100 mg/kg L-DOPA i.p. 1·5 hr before decapitation. Pieces from the oxyntic gland area were frozen to the temperature of liquid nitrogen, freeze-dried, exposed to formaldehyde gas at +80° for 1 hr for the histochemical demonstration of catecholamines and 5-HT,[11–13] embedded in paraffin *in vacuo*, sectioned transversally at 6 $\mu$ thickness, and mounted for fluorescence microscopy. For further technical details, see Falck and Owman.[14] Five untreated rats were used for the histochemical demonstration of histamine according to the *o*-phthaldialdehyde (OPT) method.[5] After decapitation, pieces from the oxyntic gland area were frozen as above, and transverse 20 $\mu$ thick cryostat sections were prepared at −25°. After drying over-night in the presence of $P_2O_5$ in a desiccator kept in the cryostat, the sections were exposed to OPT fumes for 90 sec, gently hydrated for 5 sec and mounted for fluorescence microscopy.[5] Fourteen rats were treated with 5 or 25 mg/kg reserpine i.p. followed 24 hr later by 100 mg/kg L-DOPA i.p.; they were decapitated after 1·5 hr. Pieces from the oxyntic gland area were frozen and sectioned transversally in a cryostat as above. The sections were first OPT-treated for demonstration of histamine, photographed in the fluorescence microscope and then, after removal of the cover-slip, exposed to formaldehyde gas for arylethylamine histochemistry and re-photographed.

*Determination of gastric histamine.* The mucosal histamine content of the oxyntic gland area was determined by fluorometry after extraction with organic solvents.[15,16] Specificity was secured by recording the excitation and emission spectra.

*Determination of gastric dopamine.* For these determinations the rats were injected with L-DOPA (see above). The mucosal dopamine content of the oxyntic gland area was determined fluorometrically,[17] after isolation by cation exchange chromatography.[18]

*Determination of gastric vitamin $B_{12}$-binding proteins.* The animals were killed by decapitation. The stomachs were taken out, cut open along the greater curvature, rinsed with 0·9% saline and placed on ice. The mucosa of the oxyntic gland area was scraped off, weighed and homogenized in 0·1 M sodium acetate–acetic acid buffer, pH 3·5, to a final concentration of 20 mg (wet weight) per ml. After centrifugation in a refrigerated centrifuge at 10,000 *g* for 10 min, aliquots (usually 5–10 $\mu$l) of the supernatant were added to 0·5 ml of the acetate buffer and mixed with 10 $\mu$l of [$^{57}$Co]labelled cyanocobalamin (approx. 20,000 counts/min). Aliquots (0·2 ml) of the mixture were

passed through a Sephadex G-25 column (length 200 mm, i.d. 8 mm). The column was washed with 0·1 M phosphate buffer, pH 7·0. Protein-bound vitamin $B_{12}$ was excluded from the gel and appeared in the first 3 ml fraction after the void volume. This fraction was collected and quantitated by gamma spectrometry. All determinations were run in duplicate. Blank values were obtained by running a parallel separation of the same amount of free vitamin $B_{12}$ and collecting the same fraction for quantitation. The blank value usually did not exceed 100 counts/min above background. Standards were provided by measuring the radioactivity of the aliquots before separation on the columns. For further details, see Håkanson *et al.*[8]

*Intestinal absorption of vitamin $B_{12}$.* The animals were deprived of food—but not water—for 48 hr and then given white bread soaked with a small volume of water, containing 0·03 μg [$^{58}$Co]cyanocobalamin (approx. 75,000 counts/min, instrumental value). The radioactivity retained in the body was established by whole-body counting immediately after, and 1, 3 and 7 days after ingestion of the isotope. Whole-body counting was performed in a Packard, Model 440, Armac Scintillation Detector.

## RESULTS

In accordance with previous observations, fluorescence microscopy of OPT-treated sections from the oxyntic gland area of the rat stomach revealed the presence of an extensive system of epithelial cells (identical with the enterochromaffin-like cells) predominating in the basal half of the mucosa and emitting an intense, characteristic OPT-induced fluorescence[19] due to their histamine content (Fig. 1a). In addition, histamine-containing mast cells with an OPT-induced fluorescence occurred close to the mucosal surface; they were also found scattered in the muscularis mucosae and in the submucosa.

Reserpine in doses of 5 or 25 mg/kg caused a marked reduction in the number of epithelial cells in which OPT-fluorescence could be induced (Fig. 1c). The fluorescence intensity of the remaining cells was the same as in untreated control animals. The OPT-induced fluorescence of the mast cells was unaffected by reserpine.

Rats receiving L-DOPA and killed 1·5 hr later were found to have accumulated large amounts of dopamine in the gastric enterochromaffin-like cells, which accordingly emitted an intense green formaldehyde-induced fluorescence (Fig. 1b). In agreement with previous observations, reserpine pretreatment 24 hr before injection of L-DOPA reduced the ability of the enterochromaffin-like cell system to accumulate dopamine. This was manifested by a marked reduction in the number of enterochromaffin-like

TABLE 1. EFFECT OF RESERPINE ON GASTRIC HISTAMINE, DOPAMINE AND VITAMIN $B_{12}$-BINDING PROTEINS

| Treatment | Histamine (μg/g) | Dopamine (μg/g) | $B_{12}$-binding proteins (counts/min/200 μg) |
|---|---|---|---|
| Controls | 60 ± 7 (5) | 18 ± 0·2 (5) | 8600 ± 825 (14) |
| Reserpine, 0·2 mg/kg | 58 ± 4 (5) | 17 ± 0·3 (5) | 7500 ± 1100 (5) |
| 1 mg/kg | 48 ± 5 (5) | 15 ± 1·0 (4) | 480 ± 210 (5) |
| 5 mg/kg | 36 ± 3 (5) | 8 ± 0·06 (4) | 310 ± 275 (4) |
| 25 mg/kg | 30 ± 3 (5) | 7 ± 0·03 (4) | 875 ± 160 (5) |

Mean ± S.E.M. (*n*).

cells detectable by fluorescence microscopy after the administration of L-DOPA (Fig. 1d). The fluorescence intensity of the cells that had accumulated dopamine after reserpine did not differ from that of the dopamine cells in the L-DOPA-treated control animals. Those enterochromaffin-like cells which retained their capacity to store dopamine after reserpine were found to be identical with the cells in which the OPT-induced histamine fluorescence persisted (Fig. 1c and d).

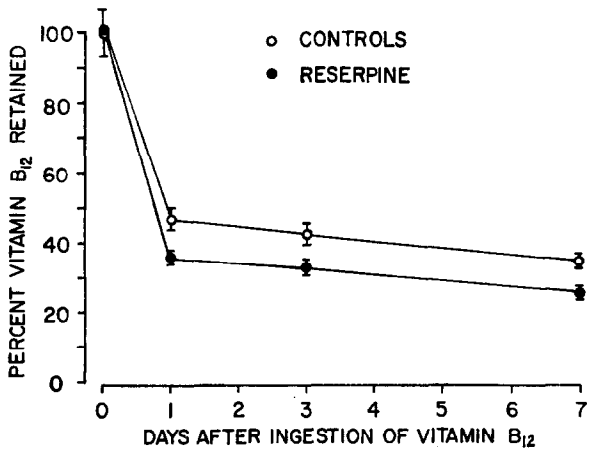


FIG. 2. Amount of radioactivity retained in the body at various time intervals after ingestion of 0·03 μg [$^{58}$Co]cyanocobalamin, expressed as per cent of the value at day 0 (75,000 counts/min). Vertical bars give S.E., $n = 6$.

The fluorescence microscopic observations on the effect of reserpine on the amine content of the enterochromaffin-like cells were confirmed by chemical determinations of gastric mucosal dopamine (1·5 hr after L-DOPA administration) and histamine. The gastric mucosal concentrations of both amines were reduced by about 50 per cent after injection of 5 or 25 mg/kg of reserpine (Table 1).

In parallel experiments reserpine was found to reduce the concentration of vitamin $B_{12}$-binding proteins in the gastric mucosa by more than 90 per cent already at a dose level of 1 mg/kg (Table 1). In contrast to this, the ability of the reserpine-treated animals to absorb orally ingested vitamin $B_{12}$ ([$^{58}$Co]labelled cyanocobalamin) was only reduced by about 25 per cent ($P < 0·01$) (Fig. 2).

## DISCUSSION

From electron microscopy it appears that the gastric endocrine cells (i.e. the system of argyrophil cells) comprise several distinct populations, distinguishable mainly on the basis of the ultrastructural characteristics of their secretory granules.[20–22] One common feature of the endocrine cells is their ability to produce and store amines such as histamine and certain arylethylamines.[3,23,24] Such endocrine cells include the enterochromaffin and enterochromaffin-like cells. The present and previous[25,26] results indicate that various populations of enterochromaffin as well as enterochromaffin-like cells can be recognized on the basis of a markedly different sensitivity to the amine-depleting action of reserpine. Thus, in the rabbit stomach, only the enterochromaffin

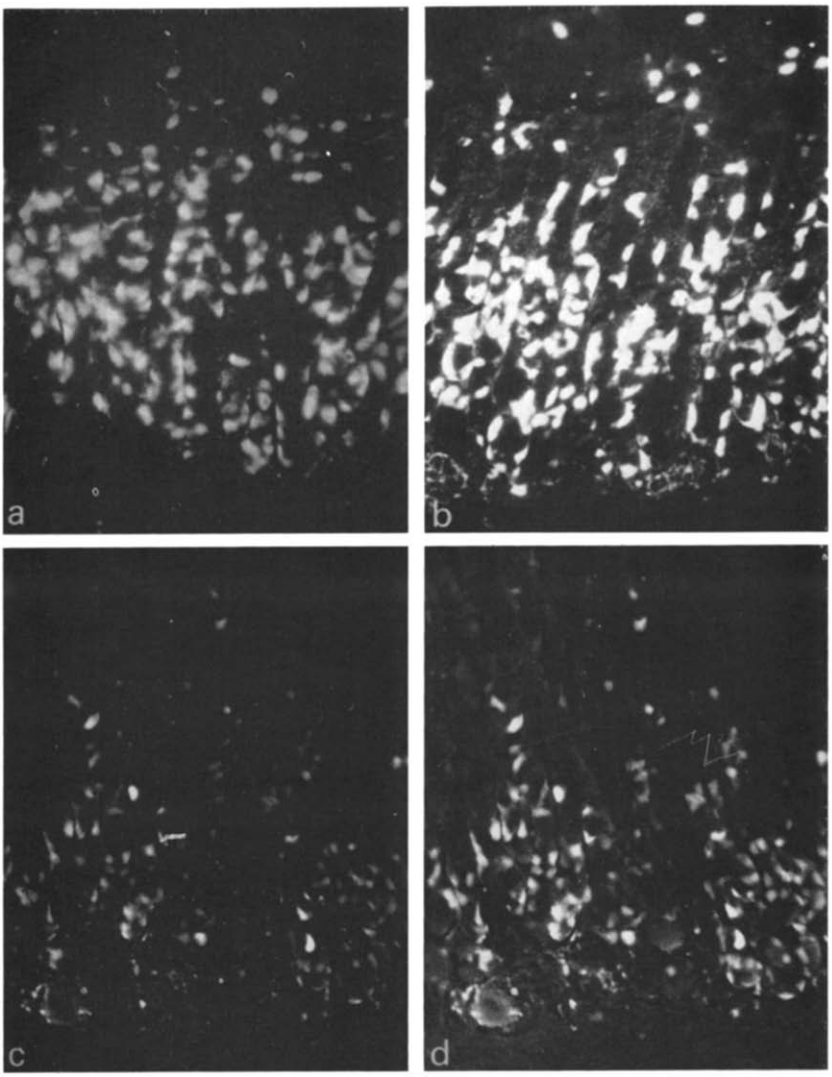


FIG. 1. Fluorescence photomicrographs of transverse sections from the oxyntic gland area of the rat stomach demonstrating the number of enterochromaffin-like cells. For phototechnical reasons the fluorescence intensities are not comparable in the different pictures. Magnification: 140×. (a) Control animal. Large number of epithelial cells emitting an OPT-induced histamine fluorescence in the basal portion of the mucosa. (b) L-DOPA-injected control animal. The system of enterochromaffin-like cells is visualized by the formaldehyde-induced fluorescence of dopamine that has accumulated in the cells. (c) Animal pretreated with 25 mg/kg reserpine and 24 hr later injected with L-DOPA. The section was first exposed to OPT showing that the number of enterochromaffin-like cells with a histamine fluorescence is markedly reduced. (d) The same section was subsequently exposed to formaldehyde to demonstrate dopamine in the cells. The number of cells that give a formaldehyde-induced fluorescence is markedly reduced, and comparison with the previous section shows that the cells accumulating dopamine are identical with those retaining histamine after reserpinization.

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cells in the pyloric gland area can be deprived of their 5-HT content by reserpine, whereas those in the oxyntic gland area remain unaffected.[25,26] Similarly, two populations of enterochromaffin-like cells can be distinguished in the oxyntic gland area of the rat: one retains the capacity to store histamine after reserpine, whereas another one loses this capacity. Reserpine did not affect gastric mast cell histamine. The results indicate the existence of three separate pools for histamine in the rat stomach mucosa.

There is recent evidence that the vitamin $B_{12}$-binding proteins of the rat stomach—which include the intrinsic factor—originate from the system of histamine-containing endocrine cells (enterochromaffin-like cells).[8] This concept is based on the strikingly similar regional and topographical distribution of the enterochromaffin-like cells and the gastric vitamin $B_{12}$-binding proteins, and on the parallel neonatal development of these cells and the amount of gastric vitamin $B_{12}$-binding proteins. The content of vitamin $B_{12}$-binding proteins in the rat stomach was drastically reduced by reserpine, which may indicate that this storage pool resides in the reserpine-sensitive population of histamine-containing endocrine cells.

The intestinal absorption of vitamin $B_{12}$ ([$^{58}$Co]labelled cyanocobalamin) was only moderately impaired by reserpine treatment (1 mg/kg, 24 hr before ingestion of the isotope). Thus, most of the intrinsic factor activity persisted *in vivo* although the level of gastric vitamin $B_{12}$-binding proteins was reduced by more than 90 per cent already at this dose level of reserpine. Two alternative explanations may be offered. Either, the intrinsic factor constitutes a minor portion of the total amount of vitamin $B_{12}$-binders in the stomach. Or, the formation and continuous release of intrinsic factor is sufficient—also under conditions of impaired storage—to satisfy the normal demands of vitamin $B_{12}$ absorption. The first alternative is not consistent with previous experiments in which the vitamin $B_{12}$-binding proteins of the rat stomach were purified (by column chromatography and gel electrophoresis) and found to be a homogenous protein probably identical with the intrinsic factor.[8]

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