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Regulation of gastric mucosal diamine oxidase activity by gastrin

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The polyamines spermidine and spermine are essential for cell proliferation. Most growth factors stimulate polyamine synthesis by inducing ornithine decarboxylase activity, which catalyzes the formation of putrescine from ornithine. Putrescine can then be utilized either for polyamine biosynthesis or may serve as a substrate for diamine oxidase (DAO), leading to formation of γ-aminobutyric acid (GABA). Growth of the oxyntic mucosa of the stomach is stimulated by feeding, by trophic hormones such as gastrin and by exogenous administration of putrescine. Conversely, fasting, as well as ornithine decarboxylase inhibition decrease oxyntic mucosal DNA synthesis. We now demonstrate that fasted rats show a high degree of [^{3}H]GABA formation from [^{3}H]putrescine in the oxyntic mucosa and that feeding or injections of gastrin, caerulein or the DAO-inhibitor aminoguanidine decrease such [^{3}H]GABA formation and, instead, stimulate formation of [^{3}H]spermidine. Moreover, gastrin injections reduced oxyntic mucosal DAO activity. Thus, oxyntic mucosal DAO activity is regulated by tropic factors and may be involved in growth regulation by controlling intramucosal putrescine metabolism.

Polyamine biosynthesis; γ-Aminobutyric acid biosynthesis; Growth factor; Aminoguanidine; Oxyntic gland mucosa; Diamine oxidase activity

1. INTRODUCTION

Numerous growth factors have been shown to stimulate ornithine decarboxylase (ODC) activity [1–4]. ODC initiates polyamine biosynthesis by catalyzing the formation of the diamine putrescine from ornithine. Putrescine is then further converted to the polyamines spermidine and spermine. A critical concentration of these molecules is necessary for mitosis, and treatment of cells with ODC inhibitors results in mitotic arrest that is reversible only upon administration of exogenous polyamines [5,6].

The growth and function of the gastric mucosa is regulated by fasting and feeding. At least part of these responses are mediated by the antropyloric hormone gastrin, released in response to feeding. Thus, administration of gastrin to starved animals stimulates DNA synthesis in the oxyntic mucosa [7]. The trophic effects of gastrin may have clinical correlates in the oxyntic mucosal hyperplasia observed in the Zollinger–Ellison syndrome [8] and in endocrine cell hyperplasia in hypochlorhydria [9,10].

Cellular putrescine levels may be regulated both by synthesis, mediated by ODC, by acetylation and by conversion of putrescine to γ-aminobutyric acid (GABA), catalyzed by diamine oxidase (DAO) (cf. [11]). As is the case with most other growth factors, inhibition of ODC blocks the trophic effects of gastrin on the oxyntic mucosa [12]. Moreover, putrescine injections also stimulate oxyntic mucosal growth [13].

Thus, intramucosal putrescine metabolism appear to be important to oxyntic mucosal growth. We therefore decided to study the role of DAO in controlling putrescine turnover. In vivo conversion of injected [^{3}H]putrescine to [^{3}H]GABA and [^{3}H]spermidine was studied under conditions of fasting, feeding and injections of trophic hormones and of the DAO inhibitor aminoguanidine. Moreover, direct measurements of oxyntic mucosal DAO activity in response to gastrin were carried out. The results show that oxyntic mucosal DAO activity is strongly controlled by trophic factors.

2. MATERIALS AND METHODS

2.1. *Animals*

Lewis rats of either sex weighing 150–200 g were fasted for 48 h and killed by a blow on the neck. 15 min to 6 h before sacrifice the animals were refed with standard rat chow or received an intraperitoneal injection of 250 μg/kg pentagastrin (Peptavlon, ICI-Pharma, Cheshire, UK), 10 μg/kg caerulein (Takus, Farmatalia Carlo Erba, Freiberg, Germany), 100 mg/kg aminoguanidine hemisulfate (Eastman Kodak, Rochester, NY, USA) or isotonic NaCl. In polyamine/GABA synthesis experiments, the rats also received an intraperitoneal injection of 1 mCi/kg [2,3-^{3}H]putrescine (30 Ci/mmol/ml, NEN) 4 h before sacrifice.

2.2. *Polyamine determination and polyamine/GABA synthesis*

The oxyntic mucosa was rinsed in cold saline and homogenized at 4°C in 0.2 M $HClO_4$. The extracted polyamines and GABA were dansylated and separated by HPLC as previously described [14]. Fractions were collected and subjected to liquid scintillation counting [14].

2.3. *Diamine oxidase assay*

DAO activity was determined by measuring formation of [^{3}H]-Δ-

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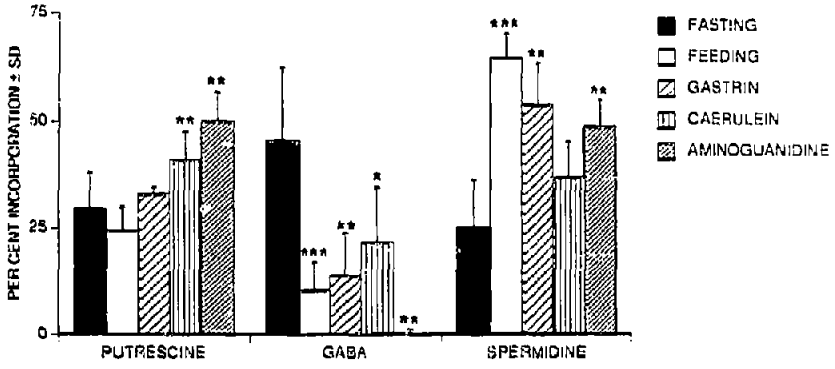

Fig. 1. Effects of fasting, refeeding, gastrin, caerulein and aminoguanidine on synthesis of [^{3}H]GABA and [^{3}H]spermidine from [^{3}H]putrescine in rat oxyntic mucosa. Results are expressed as per cent radioactivity (± S.D.) of total in putrescine, GABA and spermidine fractions separated by HPLC. ***P<0.001, **P<0.01 and *P<0.025, compared to fasted rats injected with saline. Numbers of rats used are indicated in Table I.

pyrroline from [^{3}H]putrescine. Oxyntic mucosa was homogenized (20% w/v) with a Potter apparatus in ice-cold 0.1 M sodium phosphate buffer pH 7.2, whereafter the homogenate was centrifuged at 2,000 × g for 20 min at 4°C. 100 μl of the supernatant was added to 100 μl 0.1 M phosphate buffer pH 7.2 containing, as substrate, 2 mM putrescine and 1 μCi [2,3-^{3}H]putrescine (30 Ci/mmol/ml, NEN) (the final specific reactivity in assay mixture was 5 μCi/μmol). The assay mixture was incubated for 1 h at 37°C, and the produced Δ-pyrroline extracted in 1 ml toluene. 500 μl of the toluene phase was subjected to liquid scintillation counting using Maxifluor (J.J. Baker Chemicals B.V., Deventer, Holland) as scintillate. In some experiments, 5 mM acetaldehyde was present in the assay mixture to prevent loss of Δ-pyrroline by aldehyde dehydrogenase activity [15]. Assay blanks were prepared by incubating samples in the presence of 0.1 mM aminoguanidine [15]. All determinations were made in triplicate.

2.4. *Protein determination*

Tissue solubilized in 500 μl 0.5 M NaOH was assayed for protein by the Lowry method [16].

2.5. *Statistics*

Significance of the results was determined by the Mann–Whitney U-test.

3. RESULTS

Starved rats were refed or injected with pentagastrin, caerulein, aminoguanidine or saline. After 2 h (4 h before sacrifice) they were injected with [^{3}H]putrescine. The amount of ^{3}H label was determined in the putrescine, GABA and spermidine pools of the oxyntic gland area. The total ^{3}H label of these three pools was not significantly affected by refeeding, pentagastrin or caerulein, compared to saline, whereas aminoguanidine increased the total ^{3}H label (Table I). The distribution of ^{3}H label in putrescine, GABA and spermidine fractions (expressed as percent of total radioactivity) was greatly affected by the treatments. Thus, in the oxyntic mucosa of 48 h starved rats, 45% ^{3}H label was detected in the GABA fraction and about 20% in the spermidine fraction. No or only very little label was found in the spermine fraction. This pattern changed significantly in refed rats (Fig. 1). Thus, ^{3}H label in the GABA fraction was now reducted to about 10% (P<0.001) and over 60% was detected in the spermidine fraction (P<0.001). As refeeding of starved rats is a potent stimulus for release of endogenous gastrin, we tested whether injections of pentagastrin to starved rats would mimic the effects of feeding. Such injections produced the same changes in the distribution of ^{3}H label in the GABA and spermidine pools (P<0.002) (Fig. 1). Moreover, injection of the gastrin/cholecystokinin analog caerulein produced similar changes, although of smaller magnitude than those seen after refeeding or gastrin injections. Injections of aminoguanidine to starved rats blocked the formation of [^{3}H]GABA and increased the formation of [^{3}H]spermidine from [^{3}H]putrescine, documenting that DAO activity was responsible for this conversion (Fig. 1).

The reduced conversion of putrescine to GABA after gastrin injections, indicated that gastrin decreased DAO activity. Direct measurements of DAO activity in the oxyntic mucosa showed that this was the case. Already 15 min after gastrin injection the activity of DAO in the oxyntic mucosa began to decrease, and after one hour, the activity was 64% of the activity in untreated control rats (P=0.005) (Fig. 2). Similar results were obtained

Table I

Total ^{3}H label in rat oxyntic mucosa after different treatments

Treatment	Oxyntic mucosa
Fasting (control)	104 ± 87 (8)
Refeeding	149 ± 105 (6)
Pentagastrin	106 ± 58 (4)
Caerulein	115 ± 109 (5)
Aminoguanidine	244 ± 45* (3)

Sum of ^{3}H label in putrescine, GABA and spermidine pools 4 h after injection with [^{3}H]putrescine. Values are means ± S.D. expressed as CPM/mg tissue. Numbers in brackets represent number of rats. 48-h starved rats were refed or injected with the different compounds 6 h before sacrifice. *P<0.05 as compared to fasted saline-injected rats.

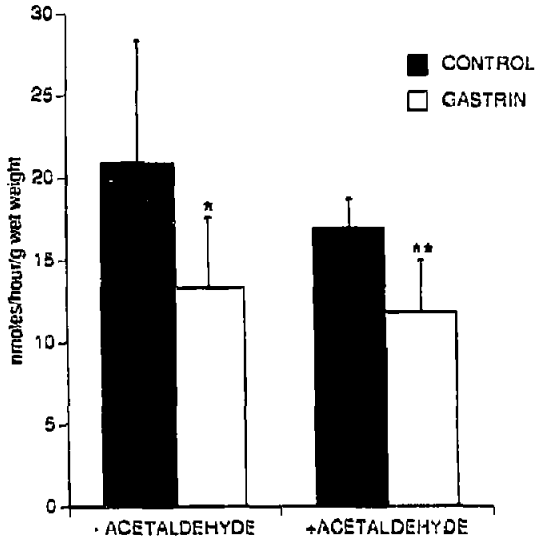

Fig. 2. DAO activity in rat oxyntic mucosa 1 h after intraperitoneal injection of gastrin (n=5) or saline (n=5). The DAO assay was also performed with surplus of acetaldehyde present in the reaction mixture to prevent possible aldehyde dehydrogenase activity from affecting the accumulation of Δ-pyrroline. $^{**}P<0.01$ and $^{*}P<0.025$.

when acetaldehyde was present in the reaction mixture to prevent possible alterations in aldehyde dehydrogenase activity from affecting the accumulation of Δ-pyrroline. Thus, one hour after gastrin injection DAO activity was decreased to 70% of the activity found in untreated controls (P=0.016) (Fig. 2). The decrease in DAO activity lasted for at least 4 h.

4. DISCUSSION

Our results clearly show that a pathway for formation of GABA from putrescine exists in the rat oxyntic mucosa. GABA can be formed both by decarboxylation of glutamate, catalyzed by glutamate decarboxylase and from putrescine, catalyzed by DAO and aldehyde dehydrogenase [11]. Previously, the putrescine pathway has been shown to exist in several tissues, including the liver, small intestine, spleen, kidney and pancreatic islets [11,14,17,18]. The fact that injections of aminoguanidine inhibit formation of [^{3}H]GABA from [^{3}H]putrescine in oxyntic mucosa strongly indicate the involvement of DAO in this conversion. The DAO catalyzed formation of [^{3}H]GABA from [^{3}H]putrescine is significantly decreased by feeding and by injections of gastrin or the gastrin/cholecystokinin analog caerulein. In addition, gastrin and aminoguanidine also cause an increased formation of [^{3}H]spermidine, possibly by increasing the amount of [^{3}H]putrescine available for spermidine synthase. Direct chemical measurements of DAO activity document a significant decrease upon gastrin injection.

DAO has previously been implicated in growth regulation [19–21] and treatment of Ehrlich ascites tumour-bearing mice with immobilized DAO has been found to inhibit tumour growth [22]. Moreover, inhibition of DAO by aminoguanidine was recently shown to enhance the intestinal adaptive response to resection [23]. Our results show that DAO activity is strongly regulated by trophic factors and indicate that DAO may play a role in oxyntic mucosal growth regulation by controlling putrescine metabolism to GABA and polyamines.

An unanswered question is whether GABA, formed by the action of DAO, has any effects itself on growth. In the central and peripheral nervous system, GABA represents a well known neurotransmitter and has been shown to have effects on gastrin and somatostatin secretion from the stomach [24].

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REFERENCES

[1] Canellakis, E.S., Kyriakidis, D.A., Rinehart Jr., C.A., Huang, S.C., Pangiotidis, C. and Fong, W.-F. (1985) Biosci. Rep. 5, 189–204.
[2] Larsson, L.-I. Progr. Histochem. Cytochem., Vol. 17, No. 4, 1988, pp. 1–222.
[3] Russell, D.H. and Durie, B.G.M., Polyamines as Biochemical Markers of Normal and Malignant Growth. Raven Press, New York, 1978.
[4] Slotkin, T.A. and Bartolome, J., In: Methods in Enzymology, Vol. 103, Academic Press, Orlando, 1983, pp. 590–603.
[5] Heby, O. (1981) Differentiation 19, 1–20.
[6] Tabor, C.W. and Tabor, H.T. (1984) Annu. Rev. Biochem. 53, 749–790.
[7] Johnson, L.R. (1976) Gastroenterology 70, 278–288.
[8] Ellison, E.M. and Wilson, S.D., in: Gastric secretion (T.K. Shnitka, J.A.L. Gilbert and R.C. Harrison, Ed.) Pergamon Press, New York, 1967, pp. 363–369.
[9] Capella, C., Polak, J.M., Timson, C.M., Frigerio, B. and Solcia, E. (1980) Ultrastruct. Path. 1, 411–418.
[10] Larsson, L.-I., Rehfeld, J.F., Stockbrügger, R., Blohme, G., Lundeqvist, G., Kindblom, L.G., Säva-Söderberg, J., Grimelius, L. and Olbe, L. (1978) Am. J. Pathol. 93, 53–68.
[11] Seiler, N. (1980) Physiol. Chem. Phys. 12, 411–429.
[12] Seidel, E.R., Tabata, K., Dembinski, A.B. and Johnson, L.R. (1985) Am. J. Physiol. 249 (Gastrointest. Liver Physiol. 12), G16–G20.
[13] Majumdar, A.P.N. and Johnson, J.R. (1987) Life. Sci. 41, 961–966, 1987.
[14] Hougaard, D.M., Høiriis Nielsen, J. and Larsson, L.-I. (1986) Biochem. J. 238, 43–47.
[15] Sessa, A., Desiderio, A.M., Baizim, M. and Perin, A. (1981) Cancer Res. 41, 1929–1934.
[16] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[17] Seilerm, N. and Dechardt, K. (1976) Neurochem. Res. 1, 469–499.
[18] Tsuji, M. and Nakajima, T. (1978) J. Biochem. 83, 1407–1412.
[19] Mennigen, R., Kusche, J. and Erpenbach, K. (1988) Biogenic Amines 5, 55–68.

[20] Metaye, T., Beudry, M. and Lalegerie, P. (1989) Int. J. Immunopharmac. 11, 629–636.
[21] Quash, G., Keolonangkhot, T., Gazzolo, L., Ripol, H. and Saez, S. (1979) Biochem. J. 177, 272–282.
[22] Mondovi, B., Gerosa, P. and Cavalieri, R. (1982) Agents Actions 12, 450–451.
[23] Rokkas, T., Vaja, S., Murphy, G.M. and Dowling, R.H. (1990) Digestion 46 (Suppl. 2), 447–457.
[24] Harty, R.F. and Franklin, P.A. (1983) Nature 303, 623–624.