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SOMATOSTATIN RELEASE FROM ISOLATED PERFUSED RAT STOMACH

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SUMMARY

Gastric somatostatin release from the isolated rat stomach was studied using a perfusion technique. Somatostatin released from the isolated perfused rat stomach was found to be identical in molecular size and immunoreactively with synthetic somatostatin. Infusion of glucagon (10^{-7}M) caused biphasic increase of gastric somatostatin release. Gastric somatostatin release was also stimulated by infusion of theophylline (10^{-3}M) and dibutyryl cyclic AMP (10^{-3}M). These results indicate the possible involvement of adenylate cyclase-cyclic AMP system in the regulatory mechanism of gastric somatostatin release.

INTRODUCTION

Immunoreactive somatostatin is demonstrated not only in the central nervous system (1, 2), but also in the pancreas (3, 4) and the stomach (3). Recently, a possible role of adenylate cyclase-cyclic AMP system in the release of pancreatic somatostatin has been suggested (5, 6). However, little is known about the release mechanism of gastric somatostatin.

The present study was performed to examine the release of immunoreactive somatostatin from perfused rat stomach and to elucidate the involvement of adenylate cyclase-cyclic AMP

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system in the regulatory mechanism of gastric somatostatin release.

MATERIALS AND METHODS

Perfusion of stomach: Stomachs were isolated from 24 h fasted male Wistar rats weighing 300-350 g by the method of Lefebvre (7). Polyethylene cannulae were inserted into the left gastric artery and the gastric vein. All other vessels were ligated and a whole pancreas was carefully excluded by ligation. After cardia was ligated, a catheter was inserted into the stomach through the pyloric ring to drain the gastric juice. 4.6 % DKRBG[1] was infused into the left gastric artery at a rate of 2 ml/min without recirculation. The perfusate was equilibrated immediately before use with a gas phase of 95 % O_2 / 5 % CO_2 at 37°C and the stomach preparation was kept at 37°C throughout the experiment. After the pre-perfusion period (20 min) when 4.6 % DKRBG was infused, 4.6 % DKRBG with or without various agents was infused into the artery for 15 min. The venous effluents were collected every 1 min in chilled tubes containing Bacitracin-Trasylol mixture (2×10^{-5}M and 1000 U/ml, respectively), frozen immediately and stored at -20°C until assayed.

Gel chromatography: 20 ml of the perfusate was pooled and shaked gently for 1 min with 2 volumes of 98 % acetone containing 0.35 N acetic acid. After centrifugation at 3500 rpm for 30 min, the supernatant was evaporated under air to remove acetone and then lyophilized. The residue was reconstituted to 1.5 ml with 0.2 N acetic acid and applied to Sephadex G-25 column (1.8 × 70 cm). Each 2 ml fraction was collected using 0.2 N acetic acid as an elution buffer, and eluates were assayed for immunoreactive somatostatin.

Radioimmunoassay: Somatostatin levels were measured by a specific radioimmunoassay with a modification of the method described by Arimura et al. (9). Specific antiserum (RA-823) to somatostatin was produced in the rabbit by repeated immunization with synthetic somatostatin (Protein Research Institute, Osaka, Japan) coupled to crystallized bovine serum albumin (Armour Pharmaceutical Co. Chicago). Antiserum RA-823 was found to have no cross-reactivity with insulin, glucagon, secretin, gastrin, motilin, VIP, substance-P, bombesin, met^5-enkephalin, ACTH, TRH, LH-RH and TSH. N-tyrosyl somatostatin was iodinated with ^{125}I-Na by chloramin-T method (8) subsequently purified on Sephadex G-25 column. The minimum detectable quantity of the assay was 10 pg/ml (Fig. 1). An intraassay and an interassay variation were 5.4 % and 8.5 %, respectively.

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[1]Abbreviations: DKRBG, dextran Krebs-Ringer bicarbonate buffer (pH 7.4) containing 5.5 mM glucose; VIP, vasoactive intestinal polypeptide; TRH, thyrotropin releasing hormone; LH-RH, luteinizing hormone-releasing hormone; TSH, thyroid stimulating hormone; db-cAMP, N^6, O^2-Dibutyryladenosine-3',5'-cyclic-phosphate, Sodium Salt.

Immunoreactive insulin was measured by radioimmunoassay with a polyethylene glycol method described previously (10). Immunoreactive glucagon was determined by radioimmunoassay with a talc adsorption technique (11) using antiserum 30 K.

N-tyrosyl somatostatin was kindly supplied from Drs. D.H. Coy and A. Arimura, V.A. Hospital, New Orleans. Antiserum 30 K was purchased from Daibetes Research Fund, Dallas. Theophylline and db-cAMP were purchased from Nakarai Chemicals, Ltd., Kyoto and Sigma Chemical Co., St. Louis, respectively. Secretin, motilin, VIP, substance-P, bombesin (generous gifts from Dr. N. Yanaihara, Shizuoka College of Pharmacy, Shizuoka), human gastrin I (a gift from I.C.I. Ltd. Macclesfield Cheshire), human ACTH (supplied from Takeda Chemical Industries Ltd., Osaka), met^{5}-enkephaline (purchased from Protein Research Institute, Osaka), TRH, LH-RH (obtained from Tanabe Pharmaceutical Co., Osaka) and TSH (purchased from Armour Pharmaceutical Co., Chicago) were of synthetic origin. Highly purified pork insulin and crystalline beef-pork glucagon were supplied from NOVO, Copenhagen and Lilly Lab., Indianapolis, respectively.

RESULTS

Three peaks of immunoreactive somatostatin were obtained from the perfusate of the stomach by gel chromatography on Sephadex G-25 column (Fig. 2). The first peak was eluted near the void volume and the main peak was demonstrated at a position of synthetic somatostatin preceded by a small second peak. Immunoreactivity of the main peak was found to be completely adsorbed to charcoal. A serial dilution curve of immunoreactive somatostatin in the main peak was quite parallel to the standard curve in the assay system (Fig. 1).

In the perfusion study, basal somatostatin levels ranged from 160 pg/ml to 217 pg/ml, which were not significantly changed during the infusion of DKRBG (mean ± SE, 183 ± 2 pg/ml) (Fig. 3). Infusion of glucagon (10^{-7}M) evoked biphasic somatostatin release with the first peak value of 1024 ± 152 pg/ml (mean ± SE) (3 min after the start of infusion) and the second of 758 ± 101 pg/ml (15 min after the start of infusion) (Fig. 3), both of which were significantly greater than the basal levels ($P < 0.01$ and $P < 0.001$, respectively). After the

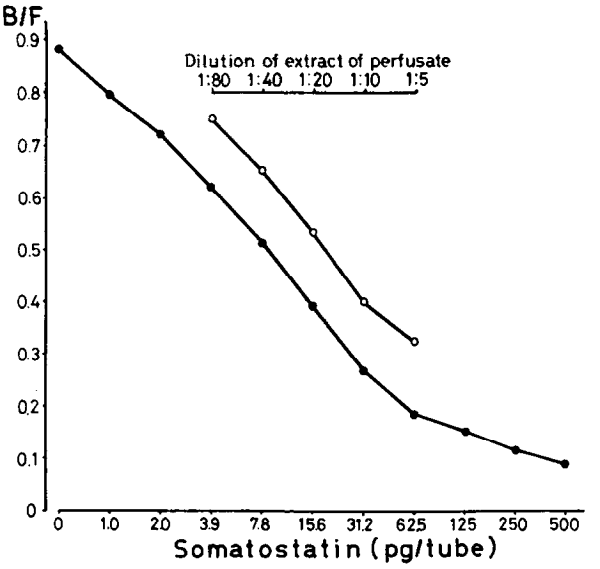

Fig. 1: Dose response curve of radioimmunoassay for somatostatin and dilution curve of the extract of perfusate from isolated rat stomach. Antiserum RA-823 was used at a final concentration with 1:8000 dilution. Non-specific binding of the assay after treatment with dextran-coated charcoal was 2.4 %. B and F show bound and free radioactivity, respectively. Standard somatostatin (●——●) and the extract from gastric perfusate (o——o) are shown.

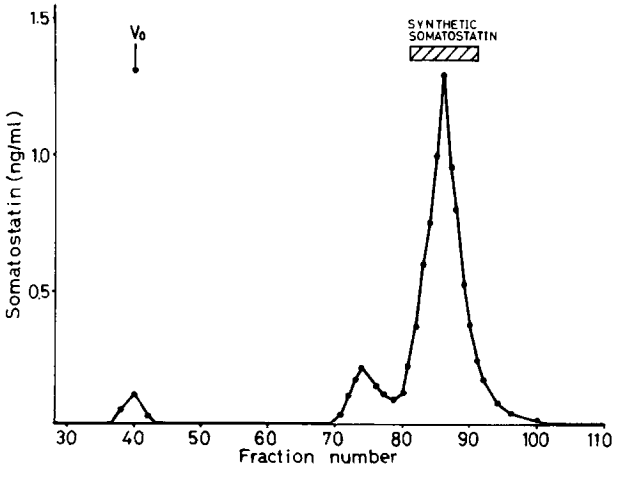

Fig. 2: Gel chromatography of the extract of perfusates from isolated rat stomach on Sephadex G-25 column (1.8 × 70 cm) in 0.2 N acetic acid at a flow rate of 25 ml/h. The column was calibrated with synthetic somatostatin (the cross-hatched bar) and dextran blue (void volume = Vo). Each fraction volume was 2 ml.

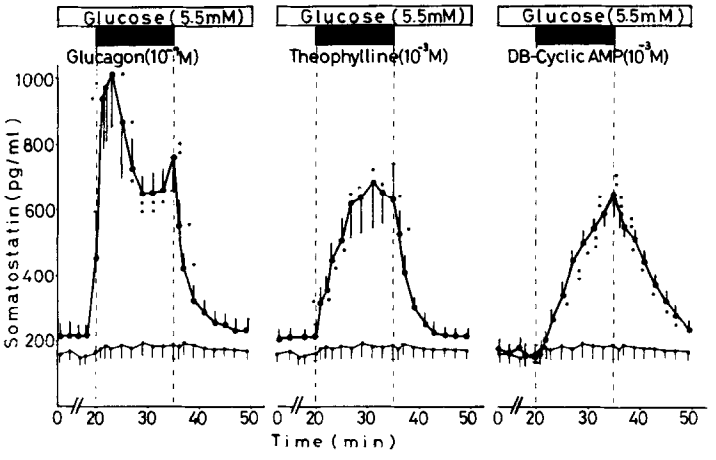

Fig. 3: Effects of glucagon (10^{-7}M), theophylline (10^{-3}M) and db-cAMP (10^{-3}M) on immunoreactive somatostatin release from isolated perfused rat stomach. All drugs were perfused for 15 min following a pre-perfusion period (20 min) as shown. Heavy solid lines (●——●) represent somatostatin responses to stimuli and light solid lines (●——●) show the control study (glucose only). Mean ± SE values are shown. Number of animals is 6 for each group. Student's "t" test was used for statistical analysis and shown as follows : * $P < 0.05$, ** $P < 0.005$ compared with the control group.

end of infusion, somatostatin levels were decreased to the pre-perfusion level within 6 min (Fig. 3). Infusion of theophylline (10^{-3}M) produced a gradual increase of somatostatin release with the peak value of 685 ± 135 pg/ml at 11 min after the start of infusion ($P < 0.01$) (Fig. 3). Infusion of db-cAMP (10^{-3}M) also elicited a gradual rise of somatostatin release with the peak value of 644 ± 34 pg/ml ($P < 0.001$) at 15 min after the start of infusion (Fig. 3).

Immunoreactive insulin and glucagon were not detected in any perfusate.

DISCUSSION

The present study revealed that immunoreactive somatostatin was released from isolated perfused rat stomach and

responded to various stimuli. Gastric somatostatin was considered to be identical in molecular size and immunoreactively with synthetic somatostatin, since the main peak of immunoreactive somatostatin was eluted in the fractions corresponding to synthetic somatostatin on Sephadex G-25 chromatography and its serial dilution curve was parallel to the standard curve.

Recent studies (5, 6) demonstrated that adenylate cyclase-cyclic AMP system might be involved in the regulatory mechanism of pancreatic somatostatin release. The present study showed that glucagon, theophylline and db-cAMP stimulated gastric somatostatin release, indicating also the possible involvement of adenylate cyclase-cyclic AMP system in the regulatory mechanism of gastric somatostatin release.

It could be considered that gastric somatostatin release might be influenced by insulin and glucagon in the stomach, since pancreatic somatostatin release is reported to be influenced by these hormones in the pancreas (12). The present study suggests, however, that it is unlikely in the case of gastric somatostatin release, since insulin and glucagon were not detectable in the perfusates of the stomach throughout the experiments. The possibility that other gastric hormones, especially gastrin, may interact with gastric somatostatin release should be elucidated in the future.

Ipp et al. (13, 14) suggested that pancreatic somatostatin might be a regulator of nutrient homeostasis. The fact that somatostatin was released from the stomach responding to various stimuli emphasizes a possible involvement of gastric somatostatin in nutrient homeostasis.

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