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Research Article

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Stimulation of Gastrin Secretion and Synthesis in Antral Organ Culture

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ABSTRACT The purpose of the present study was to examine stimulation of gastrin release and the synthesis of gastrin directly by measurement of incorporation of [^{3}H]tryptophan into gastrin in rat antral mucosal explants maintained in organ culture. Gastrin synthesis and secretion were assessed simultaneously at intervals over the 24-h duration of explant culture. Antral mucosal explants from fed female Wistar rats (4–5 wk, 100–150 g) were cultured at 37°C (95% O_2/5% CO_2) in medium containing 70% Trowell-T8 and 10% NCTC-135 without unlabeled tryptophan, 10% dialyzed fetal calf serum and [^{3}H]tryptophan (100 μCi/ml). Antral tissue was harvested at regular intervals during 24-h culture periods. Incorporation of [^{3}H]-tryptophan into immunoreactive gastrin was determined by techniques utilizing double-antibody immunoprecipitation. Antral tissue protein synthesis was assessed by measurements of incorporation of [^{3}H]tryptophan into tissue protein of cultured antral explants. In paired experiments, gastrin synthesis and secretion in the presence of dibutyryl cAMP (DBCAMP) were compared to those observed under control conditions. Gastrin and protein specific activity progressively increased with time. Gastrin specific activity at 30 min increased from 3.3±0.5 (SEM) to 55.2±10.6 fmol [^{3}H]tryptophan/pmol gastrin (or from 1.57±0.48 to 26.28±5.05 pmol [^{3}H]tryptophan/μg gastrin) at 24 h: specific activity of antral tissue protein at 30 min increased from 33.6±8.4 to 1,660±236 fmol [^{3}H]tryptophan/μg protein at 16 h. Culturing of explants for 4 h in the presence of cycloheximide (100 μg/ml) inhibited both gastrin synthesis and protein synthesis by greater than 90 and 95%, respectively. DBCAMP (10 mM) significantly increased both the synthesis and secretion of antral gastrin when compared with control cultured explants. Results of these experiments provide direct demonstration of gastrin synthesis by rat antral mucosal explants in organ culture, indicate that both gastrin and total antral protein synthesis are inhibited by cycloheximide, and demonstrate DBCAMP-induced stimulation of both gastrin synthesis and secretion, suggesting the potentially important role of cyclic AMP in gastrin cell function.

INTRODUCTION

Modern gastrointestinal endocrinology became firmly established as a scientific discipline in 1964 when Gregory and Tracy isolated pure gastrin from hog antral mucosa (1). These investigators, in collaboration with Kenner and co-workers (2), demonstrated that gastrin was a polypeptide and identified the amino acid composition and sequence of gastrin, thus permitting subsequent synthesis of the hormone (3). Immunohistochemical techniques have been utilized to localize cells that contain gastrin (G cells)[1] in the pyloric glands of the antrum and the proximal duodenum (4). The development of a radioimmunoassay for gastrin has provided a sensitive and specific method for the measurement of gastrin both in tissue extracts and in body fluids (5–7). Molecular heterogeneity applies to gastrin, as for other peptide hormones, such as insulin, parathyroid hormone and glucagon, each of which exists in more than one molecular form. Steiner et al. have demonstrated incorporation of radiolabeled amino acids into insulin (8), and have established the precursor relationship of proinsulin to insulin in cultured isolated islets of Langerhans (9). Glucose and the adenylate cyclase-cyclic nucleotide system have been shown to be important interrelated factors in stimulating release and synthesis of insulin (10, 11). In vitro organ tissue culture techniques have been employed to

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[1] *Abbreviations used in this paper*: DBCAMP, dibutyryl cyclic AMP; G cell, gastrin cell; G-17, human gastrin I; G-34, purified human big gastrin I.

study various functional and morphological characteristics of stomach (12), small intestine (13), and colon (14).

The purpose of the present investigation was to examine directly stimulation and inhibition of gastrin synthesis and gastrin secretion by rat antral mucosal explants in an in vitro system employing organ culture techniques. The incorporation of [^{3}H]tryptophan into gastrin and antral tissue protein was studied as a function of time, and the effects of cycloheximide on gastrin synthesis and antral tissue protein synthesis were determined. In addition, the effects of the dibutyryl derivative of cyclic AMP on gastrin synthesis and secretion were examined.

METHODS

Organ culture techniques. Antral mucosa was obtained from female Wistar rats between 4 and 5 wk of age and weighing 100–150 g. Nonfasting rats were sacrificed by cervical dislocation. The abdominal cavity was opened by a midline incision under sterile conditions. A 3-mm ring of antral tissue was excised and opened along the greater curvature. The excised antral tissue was then washed three times in ice-cold Hanks' balanced salt solution (Grand Island, Biological Co., Grand Island, N. Y.). Residual rat chow adherent to the mucosa was removed by gentle scraping with a sterile glass slide. Antral mucosa was dissected from the muscularis with a no. 15 scalpel blade under the dissecting microscope. Antral explants measuring to 1–2 mm^3 were prepared from antral mucosa and immediately transferred to the organ culture system. The operative time in preparing the antral explants was approximately 20 min.

Sterile plastic culture dishes (Falcon Plastics, Div. of Bio-Quest, Oxnard, Calif.) measuring 55 mm in diameter were employed in the culture of antral explants. Four organ culture grids of stainless steel mesh (Falcon Plastics) were placed in each dish. 0.3–0.4 ml of medium was delivered beneath each grid. While explants were being prepared, culture dishes with grids and medium were kept on ice. One antral explant was placed on each grid and oriented with the mucosa up and edges flat. Coverage of explants by culture medium was maintained by capillary action. The culture dishes were covered and placed inside a glass petri dish containing cotton saturated with 0.85% NaCl. Each covered petri dish was then placed inside a Torbal cannister (Torsion Balance Co., Clifton, N. J.) and gassed for 20 min with 95% O_2/5% CO_2 and the cannister was then sealed. The Torbal cannister containing the organ culture system was maintained at 37°C. Antral explants were cultured for intervals ranging from 30 min to 24 h. The four explants from a single experiment were harvested and washed three times in ice-cold Hanks' solution, blotted dry, and rapidly frozen on dry ice. Harvested explants were stored at −20°C until processed. Culture medium from each experiment was stored in a similar manner. Unless specifically noted, four to eight explants from a single experiment were processed at each incubation interval under the media conditions detailed below.

The basal culture medium contained 80% Trowell-T8, 10% NCTC-135, and 10% dialyzed fetal calf serum (all purchased from Grand Island Biological Co.). To each milliliter of medium 100 U of penicillin and 100 μg streptomycin were added. Culture medium was prepared under an ultraviolet hood. To assess gastrin synthesis by rat antral mucosal explants in organ culture, the basal culture medium was modified to contain 70% Trowell-T8 without tryptophan, 10% NCTC-135 without tryptophan, 10% dialyzed fetal calf serum, and 10% [^{3}H]tryptophan (100 μCi/ml medium). Antral explants were cultured in control medium containing [^{3}H]tryptophan and subsequently harvested in the manner as previously described. The culture medium used for these experiments was prepared specifically to minimize the presence of unlabeled tryptophan. This was accomplished by the use of tryptophan-free culture medium and fetal calf serum which was dialyzed to remove low molecular weight substances including amino acids. Tritum-labeled tryptophan, at 100 μCi/ml, was added to the medium. L-[G-3H]Tryptophan (7 Ci/mmol), with a radiochemical purity of 99% (New England Nuclear Boston, Mass.) was prepared in sterile water.

Analytical procedures. By boiling explants in 2.5 ml deionized water for 20 min antral gastrin was extracted. After centrifugation at 1,000 *g* for 30 min at 4°C, the supernate containing extracted gastrin was removed and the sediment was preserved in 0.01 M potassium phosphate buffer, pH 7.4, for protein determination. An aliquot (1.5 ml) of each gastrin extract was applied to a Sephadex G-50 superfine gel filtration column (90 × 1.5 cm) and eluted with 0.02 M barbital buffer, pH 8.4, at 4°C. Elution volume characteristics were determined for the void volume (dextran blue), salt peak (^{125}I-Na), and for purified human big gastrin I (G-34) and synthetic human gastrin I (G-17). G-34 gastrin was a kind gift of Professor R. Gregory, University of Liverpool, Liverpool, England. G-17 was purchased from Imperial Chemical Industries, Ltd. Cheshire, England. The elution volume profile of the Sephadex G-50 column for ^{3}H-radioactivity in the gastrin extract was also determined: an aliquot (0.1 ml) of each eluted fraction was added to 4 ml of Instagel (Packard Instrument Co., Inc., Downers Grove, Ill.) and counted in a Packard liquid scintillation spectrometer, model 3375 (Packard Instrument Co., Inc.). The counting efficiency of the liquid scintillation counter was determined by the method of internal standardization. Gastrin contents of individual eluted 2-ml fractions were measured by radioimmunoassay (15). The five eluted 2-ml fractions containing the immunoreactive gastrin peak were pooled and gastrin concentrations were remeasured by radioimmunoassay.

Incorporation of [^{3}H]tryptophan into gastrin was determined by techniques utilizing double-antibody immunoprecipitation. After separation by Sephadex G-50 column chromatography pooled fractions containing gastrin were incubated for 72 h at 4°C with rabbit antibodies to synthetic human gastrin I (2–17). The gastrin-antigastrin complex was then precipitated by the addition of goat antibody to rabbit IgG. After the addition of the second antibody, samples were incubated for 24 h at 4°C. The supernates were removed after centrifugation at 1,000 *g* for 55 min at 4°C and the precipitates were solubilized by the use of a toulene-based tissue solubilizer (NCS, Amersham/Searle Corp., Arlington Heights, Ill.). The solubilized immunoprecipitates were then added to 10 ml of liquid scintillation fluid (toluene 100 ml; PPO, 600 mg, POPOP, 7.5 mg) and counted in a liquid scintillation counter. The specific activity of [^{3}H]gastrin was calculated by dividing the radioactivity of [^{3}H]gastrin by gastrin concentration and was expressed as femtomoles of [^{3}H]tryptophan incorporated per picomole of gastrin. Gastrin specific activity was compared to identically prepared duplicate controls incubated with normal rabbit serum in place of rabbit antiserum to gastrin. When the double-antibody procedure was performed on column elution fractions other than those containing immunoreactive gastrin,

there was no difference in the gastrin antibody precipitated radioactivity compared with radioactivity in control precipitates.

Antral tissue protein specific activity was assessed by measuring the incorporation of [^{3}H]tryptophan into tissue protein of culture antral explants obtained after extraction of gastrin. Residual tissue sediments were homogenized with a Teflon homogenizer in 1 ml of chilled 0.01 M potassium phosphate buffer, pH 7.4. After the addition of equal volumes of 20% trichloroacetic acid (TCA), the homogenized sediments were kept at 4°C for 24 h and then centrifuged at 1,000 *g* for 30 min at 4°C. The pellets were washed once with 10% TCA and twice with chloroform-methanol (1:1 by volume) and then taken to dryness. The resultant protein-containing material was dissolved in 2 ml of 1 N NaOH. Aliquots of this solution were taken for protein measurement by the Lowry method (16) and for the determination of protein-specific activity by counting in a liquid scintillation spectrometer. The method for determining incorporation of [^{3}H]tryptophan into antral tissue protein represents a modification of the technique described by Kagnoff et al. (17). The specific activity of antral tissue protein was expressed as femtomoles of [^{3}H]tryptophan per micrograms of protein.

Gastrin secreted into the culture medium with time was measured by radioimmunoassay while the specific activity of gastrin in antral explants was assessed simultaneously by the above described methods. Gastrin concentrations in tissue culture media are expressed as nanograms of gastrin per milligrams of explant tissue protein.

Experimental design. The direct and simultaneous demonstration of gastrin synthesis and secretion by isolated, cultured antral mucosal explants was studied under control conditions with media containing [^{3}H]tryptophan. Changes in antral explant gastrin and protein specific activity with time were assessed using control media and media containing cycloheximide (Calbiochem, San Diego, Calif.). To assess the effect of the dibutyryl derivative of cyclic 3′,5′-AMP on gastrin secretion and synthesis, paired experiments were performed under control media conditions and with media containing dibutyryl cyclic AMP (DBCAMP, Sigma Chemical Co., St. Louis, Mo.). Additional control experiments were performed with 1 and 10 mM butyric acid (Sigma Chemical Co.).

Histology. Representative antral explants were taken at the specified incubation intervals from experiments performed under the above culture conditions and were examined by light microscopy. Specimens were placed in 10% neutral buffered formalin, dehydrated in increasing concentrations of ethyl alcohol, and then embedded in paraffin. Properly oriented speciments were then sectioned and stained with hematoxylin and eosin and examined microscopically.

Statistical analysis. Values are represented as the mean ± SEM. Data were analyzed by the linear regression of grouped data with analysis of variance (ANOVA). Linear regression lines were obtained using least squares analysis of group data. ANOVA was used to detect differences from linearity, significance of regression and differences between lines. Statistical significance was assigned if $P < 0.05$.

RESULTS

Elution characteristics of gastrin and ^{3}H-radioactivity in antral gastrin extracts after gel chromatography fractionation. The distribution and concentra-

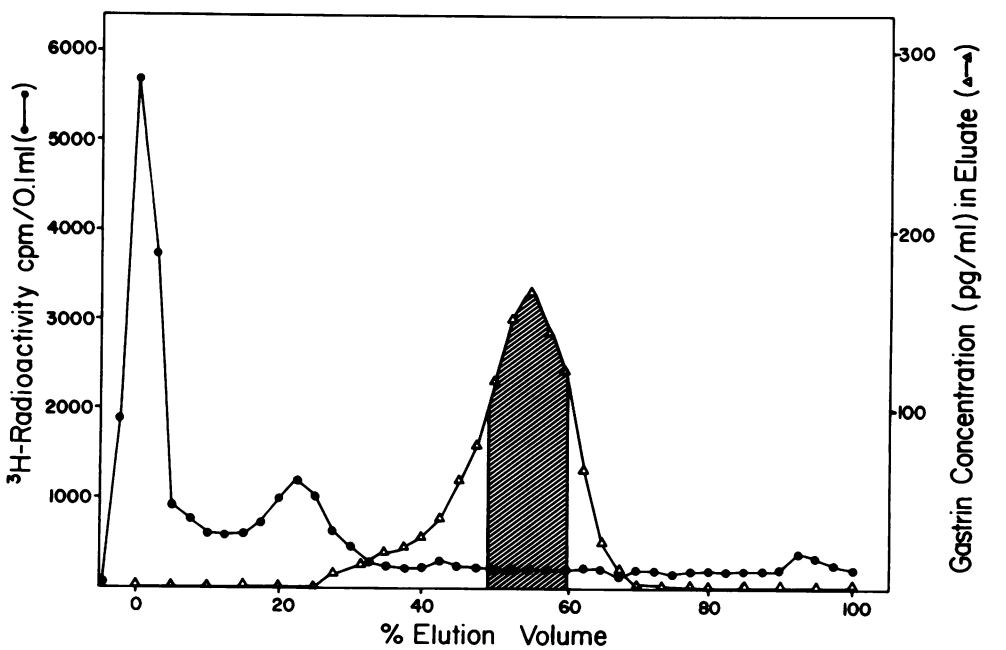

FIGURE 1 Representative elution volume characteristics of immunoreactive gastrin (△) and tritium radioactivity (●) in gastrin extracts of cultured antral explants separated by Sephadex G-50 superfine gel filtration. Elution volume is expressed as percent between the void volume and salt peak. The hatched area represents eluted fraction pooled for immunopurification of gastrin.

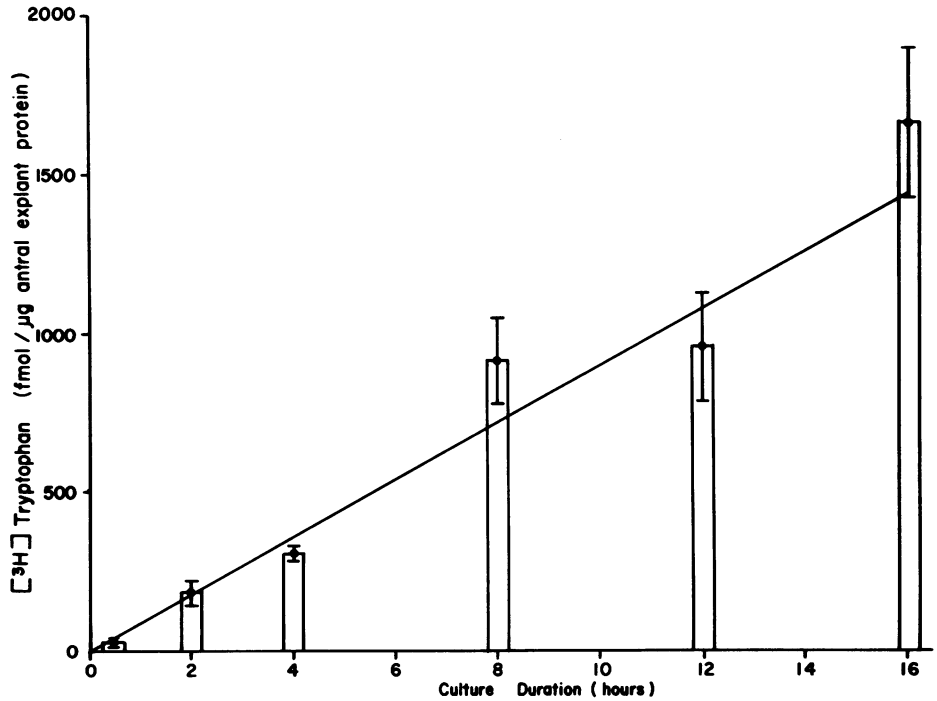

FIGURE 2 Incorporation of [^{3}H]tryptophan into tissue protein in cultured antral mucosal explants. The bars represent mean values for 4–12 experiments; the vertical lines indicate ±1 SEM. The solid line in this and subsequent figures represent a linear regression line.

tion of immunoreactive gastrin and ^{3}H-radioactivity in antral explants cultured with media containing tritium-labeled tryptophan were assessed by Sephadex G-50 superfine column chromatography. The major component of immunoreactive gastrin eluted in a single peak with an elution volume of approximately 55%, with a small leading shoulder (Fig. 1). The majority of tritium radioactivity eluted with the void volume, with a lesser peak preceding the elution volume for gastrin and a small peak in the region of the salt peak (Fig. 1).

TABLE I

Effects of Cycloheximide on [^{3}H]Tryptophan Incorporation into Antral Tissue Protein and Gastrin

Cycloheximide	[^{3}H]Tryptophan incorporation into antral tissue protein		into gastrin	
µg/ml	*fmol/µg*	*% inhibition*	*fmol/pmol*	*% inhibition*
0	186.4±28.5	—	7.0±2.1	—
100	9.3±0.6	95.0	0.69±0.05	90.2
500	11.8±2.1	93.6	0.53±0.04	92.4
1,000	10.6±0.8	94.2	0.35±0.02	95.0

In each experiment three explants were incubated individually for 4 h at 37°C as described in Methods. Cycloheximide was added at 0 time of incubation. Each value represents the mean ± 1 SEM.

Incorporation of [^{3}H]tryptophan into antral tissue protein. The specific activity of tissue protein in antral explants, cultured under control conditions with media containing tritium-labeled tryptophan, increased progressively with time (Fig. 2). Protein specific activity increased from 33.6±8.4 fmol at 30 min to 1,660±236 fmol [^{3}H]tryptophan/µg protein at 16 h. During the 16-h incubation period the mean rate of incorporation of [^{3}H]tryptophan into antral tissue protein was 102.6 fmol [^{3}H]tryptophan/µg per h. The effects of various concentrations of cycloheximide (from 100 to 1,000 µg/ml) on [^{3}H]tryptophan incorporation into explant protein were examined. The incorporation of tritium radioactivity into protein was inhibited by 95% when antral explants were cultured with cycloheximide (100 µg/ml) for 4 h (Table I). The histologic appearance of explants cultured for 4 h in the presence of cycloheximide at 100-, 500-, and 1,000-µg/ml concentrations did not differ from explants cultured for a similar duration in control media.

Gastrin synthesis. When rat antral explants were cultured with medium containing [^{3}H]tryptophan, there was a progressive increase in the incorporation of radioactivity into antral gastrin for periods up to 24 h (Fig. 3). Gastrin specific activity increased progressively from 3.3±0.5 fmol at 30 min to 55.2±10.6 fmol [^{3}H]tryptophan/pmol gastrin at 24 h. During the 24-h incubation period the mean rate of incorporation

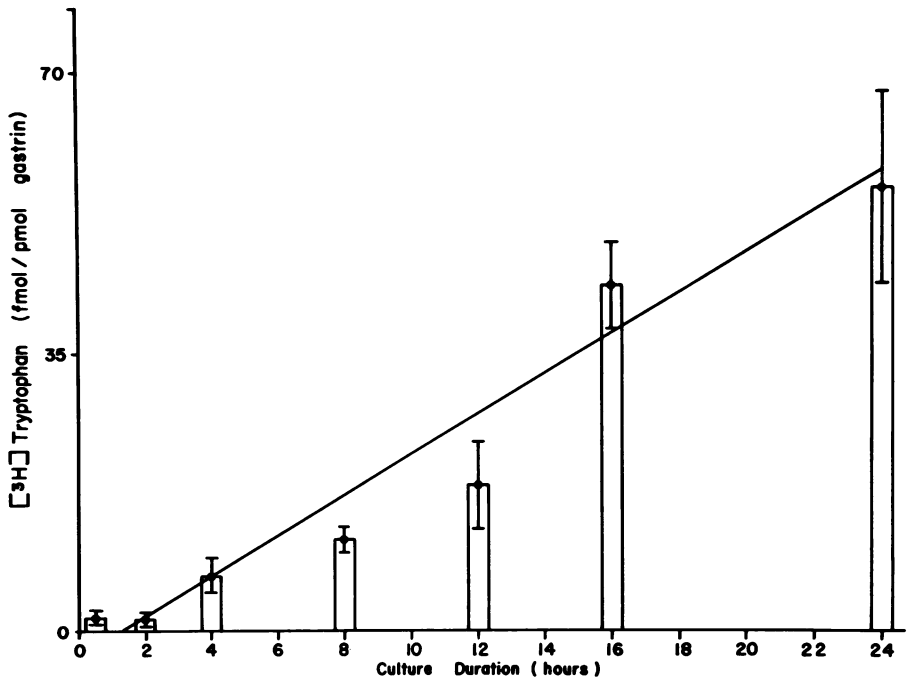

FIGURE 3 Incorporation of [^{3}H]tryptophan into gastrin in antral mucosal explants in organ culture. The bars represent mean values for 4–12 experiments; the vertical lines indicate ±SEM.

of [^{3}H]tryptophan into gastrin was 2.6 fmol [^{3}H]tryptophan/pmol gastrin (or 1,209 fmol [^{3}H]tryptophan/μg gastrin) per h. To confirm that the progressive increase in gastrin specific activity represents gastrin synthesis, experiments were performed to inhibit peptide and protein synthesis. Culturing of explants for 4 h in the presence of cycloheximide (from 100 to 1,000 μg/ml) resulted in greater than 90% inhibition of [^{3}H]tryptophan incorporation into gastrin. (Table I).

The effect of DBCAMP on gastrin secretion and synthesis by antral explants. To assess the effects of the dibutyryl derivative of cyclic AMP on gastrin secretion and synthesis by antral explants, paired experiments were performed comparing results obtained with explants cultured with control media with those obtained with media containing 10 mM DBCAMP. The concentration of gastrin in control culture media increased gradually with time, however, gastrin secretion into the medium was significantly stimulated by incubation in the presence of 10 mM DBCAMP when compared with control ($P < 0.005$) (Fig. 4, Table II). Gastrin synthesis was measured simultaneously with measurements of gastrin secretion in paired experiments performed with control media and with 10 mM DBCAMP. [^{3}H]Tryptophan incorporation into gastrin was stimulated significantly by 10 mM DBCAMP over the 24-h period of observation when compared with control ($P < 0.005$) (Fig. 5). Dose-response experiments were conducted with antral explants cultured for 16 h to examine the effects of progressively increasing concentrations of DBCAMP (from 2.5 to 40 mM) on gastrin secretion, gastrin synthesis, and protein synthesis. Maximum stimulation of gastrin secretion, gastrin synthesis, and total antral tissue protein synthesis was achieved by incubation with 10 mM DBCAMP (Fig. 6). Additional experiments were performed with butyric acid to determine possible stimulatory effects of this potential cleavage product of DBCAMP metabolism on gastrin secretion and synthesis. In the presence of 1 and 10 mM butyric acid gastrin secretion and gastrin and protein synthesis were not stimulated, but rather were reduced when compared with control values from paired experiments (Table III).

As an additional control for the specificity of gastrin synthesis as demonstrated by immune precipitation, experiments were performed in which tritium incorporation measurements were compared in the presence and in the absence of greater than 100-fold excess nonradiolabeled gastrin. In these experiments pooled Sephadex G-50 column fractions containing immunoreactive gastrin were incubated, as previously described, in the absence and in the presence of 2 μg of human gastrin I (greater than 100-fold excess of gastrin). Experiments were performed in duplicate upon the pooled gastrin fractions obtained from explants cultured both in control media and in media containing 10 mM DBCAMP for 0.5, 8, 16, and 24 h. The inclusion

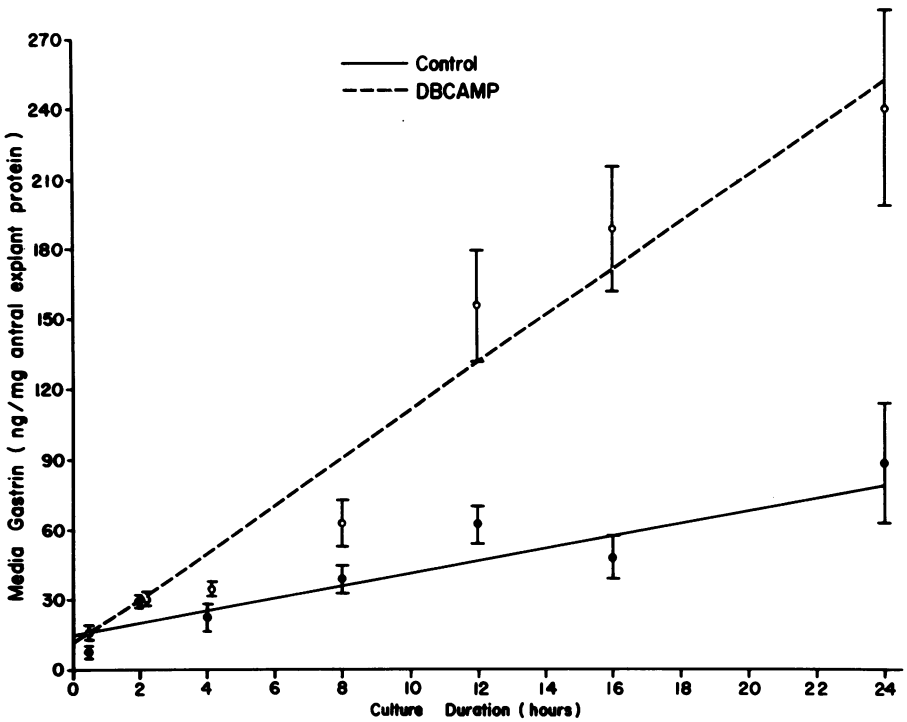

FIGURE 4 Media gastrin concentration under control media conditions and with media containing 10 mM DBCAMP. Gastrin release into the culture media was significantly stimulated by DBCAMP when compared to control ($P < 0.005$). The circles represent the mean values for control (●) and DBCAMP (○) for 4–12 experiments; the vertical lines indicate ±1 SEM. The solid and broken lines represent linear regression lines.

of excess unlabeled gastrin completely abolished detectable incorporation of [^{3}H]tryptophan into immunochemically purified gastrin. The inhibition of [^{3}H]-tryptophan into immunochemically purified gastrin was 101.5±8.1% with control media and 98.2±5.0% in the presence of DBCAMP. These results demonstrate that unlabeled gastrin displaces radioactivity ([^{3}H]tryptophan) bound by the gastrin antiserum, thus indicating the specificity of the immune precipitation incorporation determinations.

TABLE II

Effect of DBCAMP, 10 mM, on Gastrin Release and Gastrin Concentration in Cultured Rat Antral Explants

Time		No. of experiments	Media gastrin	Explant gastrin	Total explant gastrin	Gastrin release of total explant gastrin
h			*ng/mg protein*	*ng/mg protein*	*ng/mg protein*	%
0.5	C	(4)	7.3±3.3	104.8±20.7	112.5±22.8	6.5±1.8
	D	(4)	16.9±5.8	194.1±27.7	211.0±28.7	8.1±2.2
8	C	(7)	40.4±6.1	104.6±29.1	144.9±35.1	32.7±4.7
	D	(4)	63.0±12.8	117.6±28.7	180.6±36.1	36.4±4.6
16	C	(14)	46.9±9.0	73.9±18.0	120.8±25.1	42.8±5.1
	D	(8)	188.2±28.4	133.3±15.6	337.0±40.5	54.7±3.0
24	C	(6)	89.0±19.0	109.2±24.1	198.2±59.4	52.8±6.6
	D	(8)	240.4±42.3	109.4±33.5	349.9±72.9	72.5±4.0

Results are given mean±SEM. Total antral gastrin is comprised of gastrin released from the antral explant (media gastrin) and gastrin extracted from the explant during the experimental procedure (explant gastrin). Antral explants were cultured in the presence of control media (C) or with media containing 10 mM DBCAMP (D). Each experiment was done with four explants.

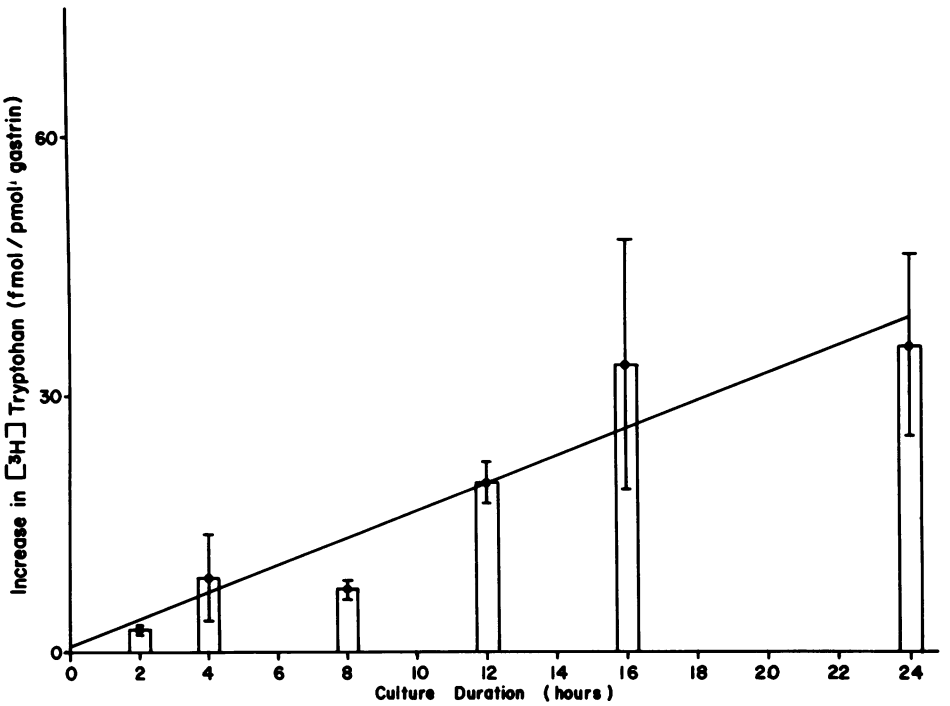

FIGURE 5 Stimulation of gastrin synthesis by DBCAMP (10 mM) as reflected by increased incorporation of [^{3}H]tryptophan into gastrin in the presence of DBCAMP when compared with control. Paired experiments were performed with control media and media containing 10 mM DCBAMP. The bars represent the mean of three to seven experiments performed at each incubation interval; the vertical lines indicated ±1 SEM.

The histological appearance of antral explants cultured for 24 h with control media or media containing DBCAMP revealed well-preserved surface epithelium and pyloric glands. By light microscopy the cellular elements of the surface epithelium appeared normal. The columnar configuration and basally oriented nuclei of the surface epithelium were maintained. The surface epithelium and pyloric glands in a small propor-

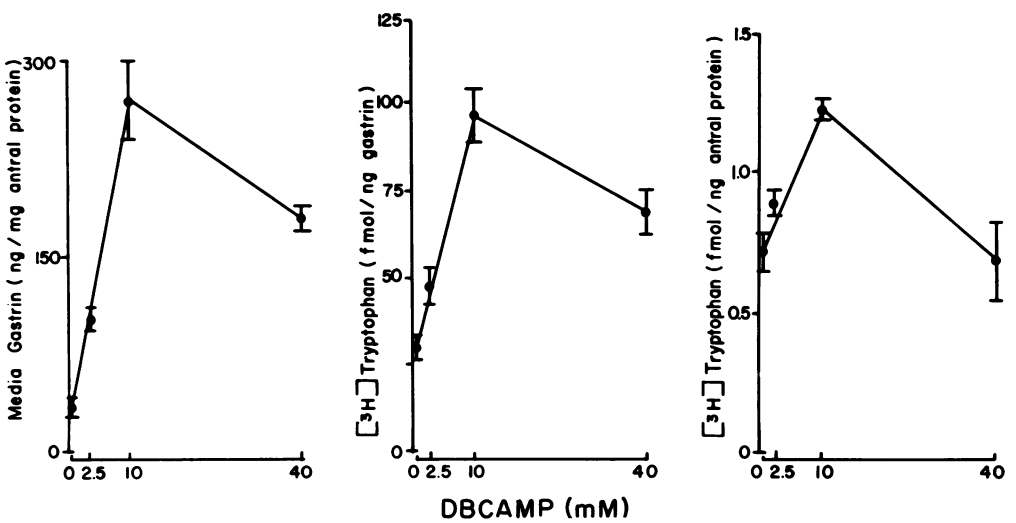

FIGURE 6 Dose response of DBCAMP on gastrin secretion, [^{3}H]tryptophan incorporation into gastrin and tissue protein in antral explants incubated for 16 h. Each point is the mean ±SEM of three experiments.

TABLE III

Effect of Butyric Acid, 1 and 10 mM, on Gastrin Release and [^{3}H]Tryptophan Incorporation into Gastrin and Protein Specific Activity

	Media gastrin	[^{3}H]Tryptophan incorporation into gastrin		into antral tissue protein	
	ng/mg protein	*fmol/pmol gastrin*	*% inhibition*	*fmol/μg protein*	*% inhibition*
Control	61.1±25.1	55.2±0.2		1,736±21.4	
Butyric acid					
1 mM	46.9±7.8	17.5±1.4	68.5	1,367±16.9	21.3
10 mM	39.6±2.9	18.7±0.9	66.1	706±47.4	59.3

Results are given as mean±SEM. In each experiment three antral explants were cultured individually for 18 h in the presence of control media or with media containing either 1 or 10 mM butyric acid.

tion of explants did show scattered areas of focal degeneration and necrosis at 24 h, which was rarely observed in explants examined at earlier time points.

DISCUSSION

The application of in vitro organ and tissue culture techniques to the study of peptide hormone secretion and synthesis has permitted investigation of endocrine cell function independent of in vivo variables, such as blood flow and hormone-hormone or hormone-neural transmitter interaction. The direct and simultaneous demonstration of peptide hormone synthesis and secretion utilizing in vitro techniques has previously been described for insulin (18), glucagon (19), and parathyroid hormone (20). Gastrin secretion, but not synthesis, by antral (21, 22) and gastrinoma tissue (23, 24) has been studied by utilizing perifusion and organ tissue culture techniques, respectively.

The results of the present studies demonstrate (*a*) direct gastrin synthesis and simultaneous gastrin secretion by isolated cultured antral mucosal explants; (*b*) that both gastrin and protein specific activities progressively increase with time; (*c*) cycloheximide almost completely abolishes gastrin and protein synthesis; and (*d*) DBCAMP increases significantly both synthesis and secretion of antral gastrin.

By examination of Sephadex G-50 elution fractions containing the immunoreactive gastrin, it was possible to assess gastrin specific activity ([^{3}H]tryptophan, femtomoles per picomoles of gastrin) in cultured explants by use of a double-antibody method for immunochemical purification of gastrin. Under control culture conditions with media containing [^{3}H]tryptophan, the specific activity of antral explant gastrin progressively increased over the 24-h period of observation. The temporally related incorporation of [^{3}H]tryptophan into gastrin establishes directly the synthesis of gastrin by cultured antral mucosa. The present studies demonstrated that both antral gastrin synthesis and secretion were stimulated by 10 mM DBCAMP; this concentration of DBCAMP, which was determined to be optimal under the conditions of these experiments, was found by other investigators to be effective in the stimulation of insulin synthesis (11).

The present studies demonstrated directly synthesis of both gastrin and total antral tissue protein by measurements of progressive increases of incorporation of [^{3}H]tryptophan into gastrin and antral protein with time. However, the rate of [^{3}H]tryptophan incorporation, expressed as femtomoles [^{3}H]tryptophan per micrograms peptide or protein per hour was much greater for gastrin than for total antral tissue protein (1,209 vs. 102.6 fmol [^{3}H]tryptophan/μg per h). Stimulation of gastrin synthesis by 10 mM DBCAMP is supported further by increases in total explant tissue gastrin (gastrin contained in the culture medium and residual gastrin in the antral mucosal explant) (Table III) when compared with explants cultured in the absence of DBCAMP.

Progressive increases in total antral tissue protein specific activity confirmed the functional viability of antral mucosa maintained in organ culture under the conditions of these experiments. Additional direct evidence that gastrin and protein are synthesized by antral mucosa in organ culture was provided by studies in which cycloheximide was included in the culture media. Cycloheximide is an antibiotic which inhibits peptide and protein synthesis at the translational level of biosynthesis by interfering with the transfer of amino acids from messenger RNA to ribosomes, thus blocking the directed polymerization of amino acids into nascent peptides and proteins (25). Gastrin and protein synthesis were almost completely abolished when explants were cultured in the presence of cycloheximide in concentration ranges from 100 to 1,000 μg/ml. It is concluded that cycloheximide-related inhibition of protein synthesis accounted for the observed decreases in gastrin and protein specific activity.

(The less likely possibility that cycloheximide may exert effects on membrane transport which are independent of its action as an inhibitor of protein synthesis cannot be excluded by these experiments [26, 27]). Histological examination of antrol explants cultured under control conditions revealed that the surface epithelium and pyloric glands were well preserved throughout the duration of culture except for occasional areas of focal necrosis in some antral explants at 24 h.

Makhlouf et al. (21) and Hayes and Williams (22), using in vitro techniques have demonstrated that antral gastrin release is stimulated by exogenous cyclic AMP or by endogenous cyclic AMP through the use of the phosphodiesterase inhibitor, theophylline. Application of the organ culture techniques described in the present study permitted direct measurement of antral gastrin synthesis and antral gastrin secretion under control media conditions and with media containing DBCAMP. Gastrin release into the culture medium was stimulated significantly by DBCAMP. In addition, DBCAMP significantly increased gastrin synthesis when compared to control cultured antral explants in paired experiments. From the results of these and previous studies (21, 22), it is possible to postulate that antral gastrin release and synthesis may be mediated, at least in part, by effects of the adenylate cyclase-cyclic AMP system on the G cell. Precise elucidation of the mechanism(s) of participation of adenylate cyclase and the cyclic nucleotides in G-cell functions will require further study.

Studies of protein biosynthetic mechanisms have shown that peptide synthesis proceeds from N-terminal to C-terminal amino acids. It has been suggested that gastrin is synthesized as the 34-amino acid big gastrin form (G-34), or as an even larger molecule, and then is converted enzymatically into the heptadecapeptide and other smaller gastrin fragments (28). Gastrin resembles several other hormones that are synthesized as larger peptides and may be converted by the action of enzymes with trypsin-like specificity to smaller, more active forms. The trypsin-sensitive region of gastrin is composed of the two consecutive basic amino acids (lysine-lysine) at the 16 and 17 position of human big gastrin (G-34). In insulin (29), glucagon (30), and parathyroid hormone (31), the trypsin-sensitive points of cleavage for conversion of large to smaller forms of the hormones are also each composed of two consecutive basic amino acids. There is no evidence for conversion of G-34 to G-17 in the circulation (32). Although not proven, conversion of G-34 to G-17 may take place in the antral gastrin cell (G cell), since most of the stored hormone is in the G-17 form. The present studies do not provide evidence relating to the possible precursor relationship of big gastrin (G-34) to heptadecapeptide gastrin (G-17).

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