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PROSTAGLANDIN E_1-STIMULABLE CYCLIC AMP FORMATION FROM RAT GASTRIC ANTRAL ORGAN CULTURE: LACK OF EFFECT ON GASTRIN SECRETION

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Explants from rat antral mucosae in organ culture were exposed to prostaglandin E_1 (2.8×10^{-5}M) for 12 h. Prostaglandin E_1 significantly stimulated the formation of cyclic AMP (pmol/mg tissue $\pm$ S.E.M.): 17.4 ± 2.5 in prostaglandin-treated samples compared to 5.5 ± 0.3 in control specimens. The increase in tissue cyclic AMP was not associated with enhanced release of immunoreactive gastrin into the culture media. Addition of isoproterenol (1×10^{-5}M) to the bathing media resulted in a 4-fold increase in antral cyclic AMP production. In contrast to prostaglandin E_1, isoproterenol exerted a marked stimulatory action on gastrin secretion during a 12 h culture period. Mucosal explants cultured for variable periods of time (6-12 h) in the presence of 8-Br-cyclic AMP (5mM) responded with a 2-fold rise in gastrin release. The results suggest that prostaglandin E_1 enhances the level of cyclic AMP in sequestered cellular compartments not functionally linked to gastrin secretory mechanisms.

Studies related to the regional gastric localization of prostaglandin responsive adenylate cyclase-cyclic AMP (cAMP) system have convincingly shown that fundic and antral mucosae respond to challenge with prostaglandin E (PGE) with a rise in cAMP formation(1).

While a wealth of information, albeit controversial, is available on the role played by prostaglandins and cAMP in the control of oxyntic cell function (2-5), the physiological relevance of PGE-stimulable cAMP formation in the gastric antrum remains conjectural.

It has been reported that exogenous cAMP stimulates gastrin release from rat antral mucosa in vitro (6). This observation provided the rationale to examine whether PGE_1 is involved in the

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control of gastrin secretion through the mediation of cAMP. The beta adrenergic agonist isoproterenol, a gastrin secretagogue (7) functionally coupled to the adenylate cyclase system, and 8-Br-CAMP served as control gastrin agonists.

MATERIALS AND METHODS

Male Charles River rats (150-200 gr) were used in all experiments. Isolated antral mucosa, dissected free of muscularis layer, was cultured according to Harty et al. (6) with minor modifications. Briefly, antral explants, measuring 1-2 mm^3, were placed luminal side up and edges flat on a wire-grid stainless steel mesh over the central well of an organ culture dish (Falcon Plastics) and cultured for variable periods of time (6-12 h). The culture medium (0.5 ml) consisted of 80% Trowell-T8, 10% NCTC-135, 10% fetal calf serum, 50 U penicillin, 50 /ug streptomycin and the phosphodiesterase inhibitor theophylline (4×10^{-3}M). The organ culture system was maintained in a metabolic incubator at 37°C in an atmosphere of 95% O_2: 5% CO_2. The following test substances were added at the specified final concentrations: 8-Br-cAMP (5×10^{-3}M); L-isoproterenol hydrochloride (1×10^{-5}M); PGE_1 (2.8×10^{-5}M). Control dishes contained the appropriate drug vehicles.

Representative sections of antral explants were prepared for routine histologic examination. Specimens collected at 6 and 12 h were fixed in neutral buffered formalin, dehydrated in increasing concentrations of alcohol and embedded in paraffin. Sections of properly oriented tissues were cut at 6 /u thickness and stained with hematoxylin and eosin.

The antral explants were weighed and the collected culture media stored at -20°C until processed for radioimmunoassay of gastrin (8). Supernatants derived from homogenates of the harvested explants were used for cAMP determination according to a competitive protein binding assay (9). L-isoproterenol, theophylline and 8-Br-cAMP were purchased from Sigma Chemical Company (St. Louis, MO); culture media and fetal calf serum were obtained from Grand Island Biological Company (Grand Island, NY). PGE_1 was a generous gift from the Upjohn Company (Kalamazoo, MI).

RESULTS

Specimens cultured and sampled at 6 and 12 h were remarkably well preserved. Surface epithelium was intact and the tubular gland structures showed distinct, cohesive cell types with a minimum of degenerative change.

PGE_1 (2.8×10^{-5}M) markedly stimulated cAMP formation from antral explants cultured for 12 h (Fig. 1). cAMP content (pmol/mg tissue $\pm$ S.E.M., n=5) was 17.4 $\pm$ 2.5 in PGE_1-treated samples compared to 5.5 $\pm$ 0.3 in control specimens cultured in PGE_1-free medium. By contrast, prolonged exposure of antral mucosa to PGE_1 had no effect on gastrin secretion (Fig. 1).

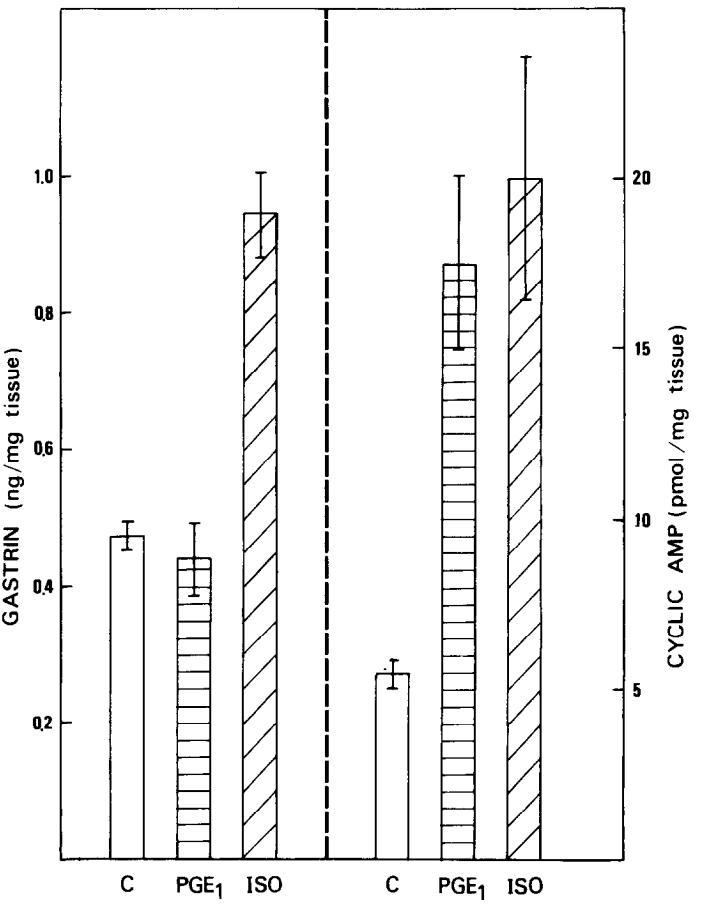

Fig. 1. Medium gastrin and tissue cAMP content of rat antral explants cultured for 12 h in hormone-free medium (C), in the presence of prostaglandin E_1 (PGE_1; 2.8 x 10^{-5}M) or isoproterenol (ISO;1 x 10^{-5}M). Vertical bars indicate means ± S.E. (n=5).

Addition of isoproterenol (1 x 10^{-5}M) to culture media resulted in a 4-fold rise in antral cAMP formation during a 12 h culture period (Fig. 1). In contrast to PGE_1, isoproterenol exerted a stimulatory action on gastrin release: immunoreactive gastrin content (ng/mg tissue ± S.E.M., n=5) in the bathing solutions was 0.94 ± 0.06 compared to 0.47 ± 0.02 in untreated control media.

Likewise for the isoproterenol-treated samples, mucosal explants cultured in the presence of 8-Br-cAMP (5mM) for variable periods of time (6-12 h) responded with a 2-fold increase in gastrin secretion into the culture media (Table 1).

TABLE 1. The stimulatory effect of 8-Br-cAMP on gastrin release from rat antral mucosae in organ culture. Means of 5 observations ± S.E.

Treatment	Cumulative Gastrin Content in Medium (pg/mg tissue)	
	6 h	12 h
None	171.6 ± 50.9	251.0 ± 67.1
8-Br-cAMP 5 mM	357.1 ± 94.4	511.7 ± 113.1

DISCUSSION

The rat antral mucosa possesses PGE_1- and isoproterenol-responsive adenylate cyclase systems. The exposure of antral explants to both agonists resulted in a marked rise in cAMP production of comparable magnitude (Fig. 1). The rise in isoproterenol-stimulable cAMP formation was associated with enhanced gastrin release into the culture media.

The salient result of the present study is that, in contrast to isoproterenol-treated specimens, gastrin secretion from antral explants was not stimulated by the addition of PGE_1 to the culture media (Fig. 1). This finding is consistent with the observation that PGE_1 is ineffective in stimulating gastrin release from perfused rat antral slices (10).

Recently, DeSchryver-Kecskemeti and Greider (11) reported an increase in gastrin secretion from fragmented rat antral glands cultured in the presence of low concentrations of PGE_1 and concluded that the prostaglandin exerts a stimulatory action on gastrin release. This assumption is, however, based on tenuous evidence, since the magnitude of the effect of low PGE_1 concentrations was of borderline significance. Moreover, high levels of PGE_1 in culture media resulted in a marked inhibition of gastrin release. In this connection it may be relevant that the increased accumulation of PGE_1 and prostanoid metabolites in hypertonic solutions bathing rat antra in vitro is associated with a significant decrease in gastrin secretion (12,13).

Although some caution is required in comparing in vitro results derived from isolated antral specimens with observations based on perfused whole stomach preparations (14) or in vivo procedures (15), the cumulative evidence strengthens the notion that prostaglandins of the E series do not play the role of gastrin secretagogues.

Exogenous cAMP was shown to exert a marked stimulatory effect on gastrin release from antral tissue in organ culture (Table 1). This finding, in conjunction with the observation that stimulation of gastrin secretion by isoproterenol is associated with a rise in antral cAMP accumulation, suggests that the cyclic nucleotide plays a mediatory role in gastrin release. If so, what is the possible explanation for the lack of an enhancing effect of PGE_1-stimulable cAMP formation on gastrin secretion?

It is well recognized that accessibility of substances to cell sites may be impeded in multicomponential explants in culture (16). This might have severely curtailed the interaction of PGE_1 with putative target cells. This contention is, however, weakened by the observation that the prostaglandin interacted with responsive antral sites with the subseqent stimulation of cAMP formation.

It could be argued also that the antral mucosa comprises heterogeneous cell populations. Conceivably, the ineffectiveness of PGE_1 in enhancing gastrin secretion may have resulted from a stimulatory effect on cAMP production in cell types other than the gastrin-producing cells (G-cells). While an unequivocal answer to this possibility awaits the availability of a homogeneous G-cell population, it is pertinent to note that most mammalian cells respond to PGE_1 challenge with a rise in cAMP formation (17,18). We therefore favor the notion that the observed changes in cAMP levels represent the summated response of all antral cells, including the G-cell population.

The possibility has been entertained that sustained challenge of cultured antral specimens with high PGE_1 concentrations render the tissue desensitized to the stimulatory effect of the prostaglandin (11). Desensitization, however, is characterized by a time-dependent diminished response to disparate agonists, including prostaglandins of the E-series (19), whereas in the present study an initial PGE_1-mediated gastrin "spurt" and subsequent decrease in gastrin secretion from desensitized antral explants were not observed.

The discrepant response of antral tissue to PGE_1 and isoproterenol, in terms of gastrin secretion, suggests intracellular compartmentalization of hormone-regulatable, cAMP-linked pathways controlling gastrin release. This notion is not based on mere conjecture, since functional compartmentalization of hormone-activated responses to cAMP have been observed in mammalian tissue (20). It is therefore reasonable to surmise that, in contrast to isoproterenol, the PGE_1-responsive pathway is not functionally coupled to gastrin secretory mechanisms but is associated with some other, yet undefined, cellular functions. The stimulatory effect of exogenous cAMP on gastrin release does not contradict the functional model proposed since occupancy by the cyclic nucleotide of sequestered compartments responsive to cAMP-linked gastrin secretagogues will be perceived by the indiscriminating G-cell as a signal for gastrin secretion.

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