NEURAL AND PARACRINE REGULATION OF GASTRIN RELEASE USING RAT ANTRAL MUCOSA IN TISSUE CULTURE
—THE EFFECT OF CARBACHOL, BOMBESIN, AND ANTI-SOMATOSTATIN ANTIBODY ON GASTRIN RELEASE—

Takeshi AZUMA, M.D., Takashi KAWAI, M.D., Hideto INOKUCHI, M.D. and Keiichi KAWAI, M.D.
Department of Preventive Medicine, Kyoto Prefectural University of Medicine, Kyoto 602, Japan

Summary

Gastrin release was significantly stimulated by the cholinergic agent carbachol at doses of 10^-4 M, 10^-5 M, and 10^-6 M. Peak stimulation was observed at 10^-5 M. Gastrin release was also significantly stimulated by bombesin at a dose of 10^-6 M and 10^-7 M atropine which abolished the effect of carbachol in stimulating gastrin release had no effect on the bombesin-stimulated gastrin release. In addition, anti-somatostatin antiserum significantly stimulated gastrin release. These findings suggest that gastrin release is regulated by cholinergic and noncholinergic neurons the latter being thought to be a bombesin-containing neuron, and that antral somatostatin exerts a continuous restraint on gastrin release by the paracrine mechanism.

Key Words: Anti-somatostatin antiserum, Bombesin, Carbachol, Gastrin release, Tissue culture.

Introduction

Regulation of gut hormone release involves multiple hormonal, neuronal, and paracrine factors. In the case of gastrin, members of the secretin-glucagon family, glucagon, secretin, and VIP, have been shown to inhibit its release. Gastrin release is also regulated by the activity of two interdependent intramural neurons. One is cholinergic; its activation causes inhibition of somatostatin release and leads to stimulation of gastrin release. The other neuron is noncholinergic and is thought to release a peptide, probably bombesin, capable of direct stimulation of gastrin release. Furthermore, somatostatin cells in human and rat antral mucosa have cytoplasmic processes that come into close contact with gastrin cells. This structural feature suggests a functional linkage between the two cell types. Infusion of somatostatin antiserum into the isolated vascularly perfused rat stomach causes a significant increase in gastrin release; the finding implies that antral somatostatin exerts a continuous restraint on gastrin release by para-
Several techniques have been used to study gut hormone release in vitro. These include mucosal slices perfused in a chamber or maintained in organ culture, oriented mucosal sheets, luminally-perfused segments of the gut, isolated vascularly-perfused organ, and suspensions of mucosal cells enriched in peptide-secreting cells. Each has advantages and disadvantages.

The purpose of the present study was to examine the regulation of gastrin release, especially neuronal and paracrine regulation, using rat antral mucosa in tissue culture.

**Materials and Methods**

**Tissue culture techniques**

The tissue culture preparation used was a modification of the technique of Harty et al. Gastric antral mucosal tissues were obtained from nonfasted female Sprague-Dawley rats (150–200 g) immediately after cervical dislocation. Antral mucosal tissues were dissected from the muscle layers and washed three times in saline containing 100 U/ml Penicillin and 100 micro g/ml Streptomycin. Antral mucosal explants (1–2 mm^3^) were transferred to tissue culture system. A single explant was placed on the tissue culture stainless steel grid and oriented with the mucosa up and edges flat. Milliliter medium (0.4–0.5 ml RPMI 1640 containing 10% fetal bovine serum, 100 U/ml Penicillin, and 100 micro g/ml Streptomycin) was delivered beneath each grid, and coverage of explants by medium was maintained by capillary action. The culture dishes were covered and placed in a CO\textsubscript{2} incubator in a humidified environment of 95% air and 5% CO\textsubscript{2} at 37°C.

**Histology**

Representative antral explants were taken at regular intervals over the duration of culture (0 min, 1 hr, 2 hr, 3 hr, 6 hr), and were examined by light microscopy. Specimens were fixed in Bouin's solution, routinely processed and embedded in paraffin. Sections of 4 micron thickness were cut and stained with hematoxylin and eosin and examined microscopically.

In addition, gastrin containing cells (G cells) and somatostatin containing cells (D cells) were examined by immunohistochemistry. For immunostaining, the unlabelled peroxidase-anti-peroxidase complex (PAP) technique, as described by Sternberger, was applied. Antibodies used were anti-human gastrin antiserum (purchased from Bioproducts, Belgium) and anti-human somatostatin antiserum (purchased from DAKO, Denmark).

**Experimental design**

After a 45 min stabilization rat antral mucosal explants were cultured for 2 hr with or without drug or serum. Media were collected at regular intervals over the duration of culture (0 min, 30 min, 1 hr, and 2 hr). The drugs or the sera which were tested alone or in combination were carbamylcholine (Sigma, St. Louis MO), bombesin 14 (Peninsula), atropine sulfate (Sigma), anti-somatostatin antiserum 1001 (generous gift from G.W. Aponte, Los Angeles, USA). Gastrin secreted into the culture medium was measured by the radioimmunoassay technique previously described using antibody 1611 (generous gift from J.H. Walsh, Los Angeles, USA). The results of assays at regular intervals were expressed as a percent change from level at 0 min in each experiment.

**Statistical analysis**

Values were presented as the mean ± SEM. Statistical significance of differences between groups of samples was assessed by Student's unpaired t-test with significance assigned values of p<0.05.

**Results**

**Histology**