EFFECTS OF EXOGENOUS AND ENDOGENOUS BOMBESIN ON GASTRIN SECRETION FROM RAT ANTRAL MUCOSA IN TISSUE CULTURE

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

Effects of exogenous and endogenous bombesin on gastrin secretion were examined using rat antral mucosa in tissue culture. Gastrin secretion was significantly stimulated by exogenous bombesin at a dose of $10^{-8}$ M. Atropine $10^{-6}$ M, which abolished the action of the cholinergic agent carbachol to stimulate gastrin secretion, had no effect on bombesin-stimulated gastrin secretion. In addition, gastrin secretion was significantly inhibited by anti-bombesin antiserum used to block the effect of endogenous bombesin by immunoneutralization. These findings suggest that the stimulation of gastrin secretion by bombesin does not involve cholinergic neural pathways and that endogenous bombesin exerts a continuous stimulation on gastrin secretion in the basal state.

Key Words: Anti-bombesin antiserum, Bombesin, Gastrin secretion, Tissue culture.

Introduction

Bombesin was originally isolated from the skin of the frog Bombina bombina, one of the most potent actions is release of mammalian antral gastrin. Bombesin-like immunoreactivity (BLI) has been demonstrated in extracts of porcine and rat gastrointestinal tissues, in especially high concentrations in the stomach. Furthermore, BLI is found in nerve fibers in the myenteric and submucosal plexuses and mucosal nerve fibers in the stomach.

In in vitro studies, stimulation of gastrin secretion by cholinergic agents was mediated in part by inhibition of somatostatin secretion. Elimination of somatostatin restraint by cholinergic inhibition of somatostatin accounts only partly for the gastrin response to neural stimulation. The residual atropine-resistant gastrin response is mediated by a noncholinergic transmitter. The most likely candidate is mammalian bombesin, also known as gastrin-releasing peptide for its ability to stimulate gastrin secretion.

The purpose of the present study is to examine the effect of exogenous and endogenous bombesin on gastrin secretion using rat antral mucosa in tissue culture.

Materials and Methods

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 μg/ml Penicillin and 100 μg/ml Streptomycin. 1–2 mm³ antral mucosal explants 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. 0.4–0.5 milliliter of media (RPMI 1640 containing 10% fetal bovine serum, 100 μg/ml Penicillin, and 100 μg/ml Streptomycin) was delivered beneath each grid. The culture dishes were covered and placed in CO2 incubator in a humidified environment of 95% air and 5% CO2 at 37°C.

The effect of gastrin secretion by exogenous bombesin was examined initially in dose-response experiments from 10⁻⁶ M to 10⁻⁹ M. The effect of gastrin secretion by endogenous bombesin was examined using anti-bombesin antiserum 1078 (generous gift from J.H. Walsh, Los Angeles, USA)¹⁰). Bombesin 14 (Peninsula) or anti-bombesin antiserum 1078 was added to the basal medium after a 45 min stabilization culture. Rat antral mucosal explants were cultured for 2 hr.

Media was collected at regular intervals over the duration of culture (0 min, 30 min, 1 hr, and 2 hr). Gastrin secreted in the culture medium was measured by a radioimmunoassay technique using antibody 1611 (generous gift from J.H. Walsh)¹¹).

Results

Effect of Bombesin on Gastrin Secretion

The dose-response effects of bombesin on gastrin secretion were examined. Bombesin significantly stimulated gastrin secretion to 198.0 ± 24.7% above 0 min levels at a dose of 10⁻⁸ M after 2 hr incubation. Doses of 10⁻⁶ M, 10⁻⁷ M, and 10⁻⁹ M bombesin also stimulated gastrin secretion but not to a significant degree (Fig. 1).

Atropine (10⁻⁶ M), which was reported to abolish the action of the cholinergic agent carbachol to stimulate gastrin secretion in a previous paper¹²), had no effect on bombesin-stimulated gastrin secretion (Fig. 2).

Effect of Anti-Bombesin Antiserum on Gastrin Secretion

Anti-bombesin antiserum was used to block the effect of endogenous bombesin by immunoneutralization. The effects of control rabbit serum and anti-bombesin antiserum 1078, diluted 1:10 with basal media, were examined.

Statistical Analysis

Values were presented as the means ± SEM. The statistical significance of differences between groups of samples was assessed by Student’s unpaired t-test with significance assigned to values of p<0.05.

![Graph](image-url)

Fig. 1. The dose-response effects of bombesin on gastrin secretion. The bars represent means ± SEM (n=8) of percent change from 0 min levels. The asterisk (*) indicates values significantly different from control (p<0.05).