A SPECIFIC GASTRIN RECEPTOR ON PLASMA MEMBRANES OF ANTRAL SMOOTH MUSCLE

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SUMMARY

Plasma membranes with a 17 fold enrichment in 5'-nucleotidase over homogenate were prepared from antral smooth muscle. A specific gastrin receptor on the plasma membranes has been demonstrated. By Scatchard analysis receptor has a K_{aff} of $2x10^{9}M^{-1}$ and a binding capacity of $5x10^{-14}$ moles/mg. of membrane protein.

INTRODUCTION

In spite of the rapid growth in the field of hormone receptors, there has been no direct characterization of gastrin to a specific plasma membrane receptor. This is because of formidable technical problems encountered in trying to obtain a plasma membrane preparation from the most obvious gastrin target organ, gastric fundal mucosa. As yet it has not been possible to obtain a pure preparation of parietal cells from the mixed population of cells of the mucosa , in adequate numbers to yield usable quantities of plasma membranes. We circumvented these problems by choosing another, more homogeneous, gastrin target organ i.e. antral smooth muscle.

Gastrin and pentagastrin (the t-Boc-β-Ala derivative of gastrin C-terminal tetrapeptide) increase antral motility in dog and man during normal digestion (1, 2, 3). Gastrin increases motility by increasing the rate of the pace setter potential; this effect of gastrin is primarily on the muscle itself, as it is not inhibited by atropine or tetradotoxin, an agent which inhibits axonal conduction (4, 5, 6).

In this report we describe the preparation of plasma membranes from dog antral smooth muscle and some characteristics of the specific gastrin receptors on those membranes.

MATERIALS AND METHODS

Materials: Synthetic human gastrin I-17 (referred to as gastrin) was obtained from Imperial Chemical Corp., U.K., (15-leu)gastrin from Research Plus Lab., pentagastrin from Vega-Fox Biochemicals, porcine monocomponent insulin and glucagon from Dr. Ronald Chance of Eli Lilly Co., human growth hormone from N.I.H. Hormone Resource and Trasylol from Dr. G. Schnells, Bayer, Germany.

Plasma Membrane Preparation: After mucosa and connective tissue were dissected away, antral muscle layer was homogenized for 35 seconds in a Waring blender with 20 volumes (w/v) of 8% sucrose - 0.02 M Tris, pH 7.4. The homogenate was filtered through 18 mesh nylon then centrifuged for 12 minutes at 5,000 g_{avg}. The supernatant was centrifuged at 1 hour at 100,000 g_{avg}. After the resulting crude microsomal pellet was washed by homogenizing and repelleting, it was homogenized in 2 ml of 45% sucrose and layered on the bottom of discontinuous gradient of equal volumes of 10, 28, 30, 32, 36 and 45% sucrose - 0.02 M Tris pH 7.4 in a 1.4 x 8.9 cm tube and centrifuged for 2 hours at 100,000 g_{avg}. Membrane vesicles recovered from interfaces were pelleted and washed in buffer appropriate to the assay being done. All above was done at 4°C.

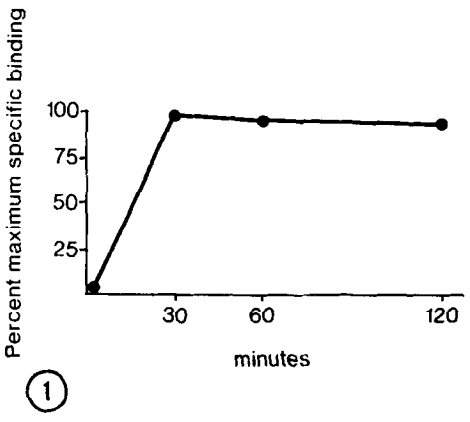
Gastrin-Receptor Binding: 80 µg of membranes were incubated with 60,000 cpm of [^{125}I]gastrin trace and varying amounts of unlabelled hormone in 0.5 ml of buffer of 148 mM NaCl, 3 mM $MgSO_4$, 2.7 mM KCl, 10 mM Tris-maleate, 0.05% gelatin with 250 units of Trasylol (a protease inhibitor) at 20°C. Membrane bound hormone was separated from solution and washed on 0.4 µ pore polycarbonate filters (Nuclepore) and filters counted in a Nuclear Chicago gamma counter. Total binding was defined as amount (determined from cpm) of [^{125}I]gastrin trace bound to plasma membranes, specific binding as difference in amount of trace bound in the presence and absence of an excess (1×10^{-6}M) of unlabelled gastrin and nonspecific binding as total minus specific binding ($p < .05$ for t test for difference of the means of cpm with 10 samples for each mean value).

Additional Methods: Biologically active mono-iodo [^{125}I]gastrin with mean specific activity of 0.7 Ci/mol was prepared as described by Stadil and Rehfeld (7,8). 5'-nucleotidase (E.C. 3.1.3.5) was assayed by method of Heppel and Helmore (9) with units of activity defined as µ mol P_i liberated /15 min. Protein was measured by method of Lowry (10).

RESULTS

Characterization of Plasma Membrane Preparation: Application of the crude microsomal preparation to a discontinuous sucrose gradient resulted in a plasma membrane enriched fraction at the 32/36% sucrose interface. Specific activity of the plasma membrane marker-enzyme 5'-nucleotidase in this fraction was 32.4 (± 2.0) U/mg as compared to 1.9 (± 0.4) in the homogenate (± S.E. for 6 preparations). This represents a 17 fold enrichment in plasma membranes over the homogenate.

Characteristics of Specific Gastrin Binding: At 20°C specific binding was found to be maximum at 30 minutes and remained constant for 2 hrs. (Fig. 1). The optimum pH for specific binding was between 6.6 and 6.8 (Fig. 2). After



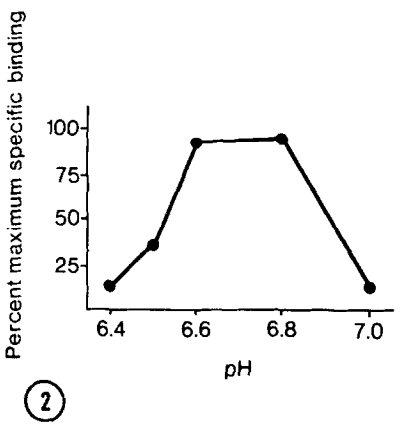


Figure 1. Time course of specific gastrin binding at 20°C.

Figure 2. Percent maximum specific gastrin-receptor binding at varying pH.

determining these steady state conditions all subsequent assays were done at pH 6.8 with an incubation time of one hour.

Under these conditions maximum specific binding with 80 μg of plasma membrane material was 0.12% of the [^{125}I]gastrin trace. Doubling or halving this membrane concentration resulted in a doubling or halving of the percent of [^{125}I]gastrin trace specifically bound, indicating that specific binding is a linear function of membrane concentration over the range of membrane concentrations used in assays.

While [^{125}I]gastrin did bind to filters, filter binding was completely nonspecific i.e. not inhibited by unlabelled gastrin. After subtracting filter binding, specific binding to membranes was found to be 53% of total membrane binding.

Fig. 3 summarizes the ability of unlabelled gastrin and other polypeptides to competitively inhibit [^{125}I]gastrin trace binding. The percent of [^{125}I]-gastrin bound decreases as the concentration of unlabelled gastrin increases, with maximal inhibition occurring at 1×10^{-7}M. Unlabelled pentagastrin is a less potent inhibitor, in keeping with its weaker biologic effect. Unrelated polypeptide hormones insulin, glucagon and growth factor show no inhibition.

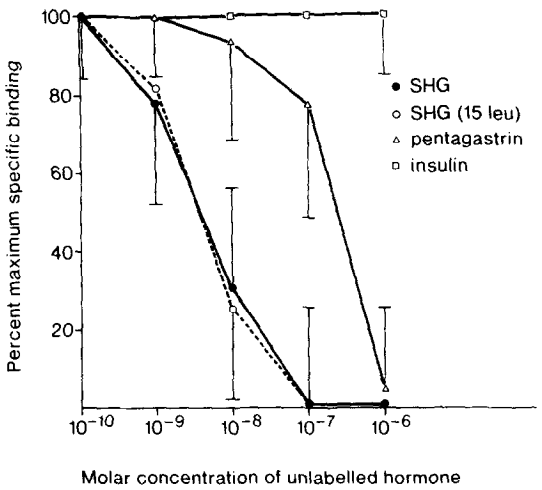


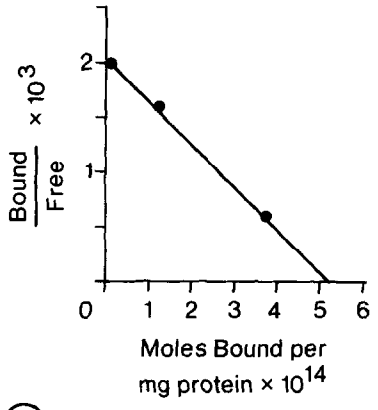
Figure 3. Competitive inhibition of $5x10^{-11}$M [^{125}I]synthetic human gastrin I-17 (± S.E.) by: a) ●—● synthetic human gastrin I-17 (SHG), b) O—O (15-leu)SHG, c) Δ—Δ pentagastrin and d)□ — □ insulin (as well as growth hormone and glucagon).

Scatchard analysis of this data (Fig. 4) shows a single receptor site with an association constant of $2x10^{9}M^{-1}$ and a binding capacity of $5x10^{-14}$ moles/mg or $5x10^{-13}$ moles/U 5'-nucleotidase.

Evidence that gastrin binding is to the plasma membrane is shown in Fig. 5. Using equal protein amounts of both a crude microsomal preparation and a purified plasma membrane fraction, a greater amount of gastrin was found to bind to the plasma membrane fraction. The greater amount of specific gastrin binding to the plasma membrane fraction was directly proportional to the greater activity of the plasma membrane marker enzyme 5'-nucleotidase.

DISCUSSION

We have demonstrated specific gastrin binding to plasma membranes of a gastrin target organ, antral smooth muscle. Purification of plasma membrane fraction was assessed by measuring specific activity of 5'-nucleotidase, shown by histochemical techniques (11) to be located predominently in plasma membranes. Ability of membranes to specifically bind gastrin was directly proportional to purification of the membrane marker.



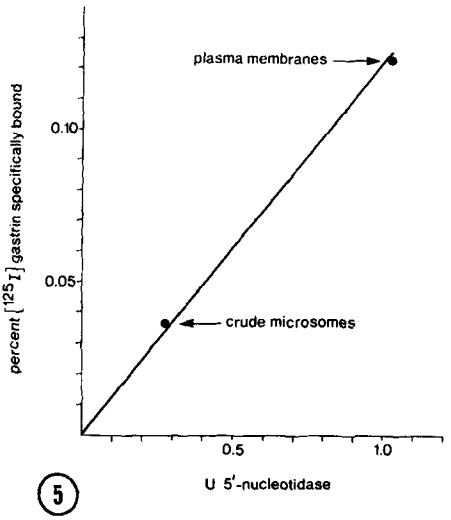


Figure 4. Scatchard plot of competitive inhibition of [^{125}I]synthetic human gastrin I-17 by unlabelled hormone.

Figure 5. Specific gastrin binding to crude microsomal membranes and plasma membranes. Abscissa shows units of 5'-nucleotidase activity in 80 µg of membrane protein.

Specific binding as a percent of total binding was lower than that which we obtained for binding of insulin to striated muscle plasma membranes (12). In those studies the binding buffer contained albumin, commonly used in [^{125}I]-polypeptide hormone binding studies precisely for the purpose of reducing non-specific binding. Since evidence exists that gastrin may bind to albumin in significant amounts (13), we were unable to use albumin in buffers in this study.

The association constant for specific gastrin-receptor binding, $2 \times 10^{9} M^{-1}$, is compatible with a physiologically significant receptor. The possibility that there is "specific" binding to the filters has been excluded (14). Additional evidence that the receptor is of physiologic importance is provided by experiments demonstrating that the gastrin fragment, pentagastrin, binds in molar proportion to its known activity in the whole animal. The mechanism by which gastrin effects the contractility of smooth muscle has yet to be elucidated.

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