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**Research Article**

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# In Vitro Action of Bombesin on Amylase Secretion, Membrane Potential, and Membrane Resistance in Rat and Mouse Pancreatic Acinar Cells

## A COMPARISON WITH OTHER SECRETAGOGUES

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ABSTRACT Bombesin caused depolarization of rat or mouse pancreatic acinar cell membrane, reduction of membrane resistance, and a steep rise in amylase output from superfused pancreatic fragments. These effects were similar to those previously described for acetylcholine, cholecystokinin, and gastrin. The dose-response curves for these three effects of bombesin were very similar, with effects being detectable at concentrations of about 30 pM and maximal effects at about 10 nM.

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## INTRODUCTION

The tetradecapeptide bombesin, that can be isolated from amphibian skin, is a powerful stimulant of pancreatic secretion in several species including man (1–4). Bombesin-like peptides are present in the mammalian gastrointestinal tract (5, 6). Bombesin appears to have a direct action on pancreatic acinar cells (3, 4) and it has been suggested that it interacts with receptor sites distinct from those reacting with acetylcholine (ACh),[1] cholecystokinin (CCK), and secretin (3, 4). ACh, CCK, and gastrin all evoke marked changes in pancreatic acinar cell membrane resistance and potential (7–10). These changes reflect increased membrane permeability to certain ions and probably represent an important step in the stimulus-secretion coupling sequence (11). In contrast, secretin has no such effect (9).

It was therefore of interest to test the effect of bombesin on the potential difference and resistance across the pancreatic acinar cell membrane and to compare possible effects with those of the other stimulants. In addition, the use of an automated high time resolution fluorometric technique for measuring amylase activity (12) has allowed a comparison between the dynamic profile of amylase output after bombesin and ACh stimulation.

## METHODS

Pancreases were removed from fasted adult mice or rats killed by cervical fracture or decapitation.

*Membrane potential and resistance measurement.* Pancreatic segments about 3 × 3 mm, always including a totally undamaged free surface area of the gland, were secured to a perspex platform placed in a perspex tissue bath (20 ml) through which a Krebs-Henseleit solution with the following composition: NaCl, 103 mM; KCl, 4.7 mM; $CaCl_2$, 2.56 mM; $MgCl_2$, 1.13 mM; $NaHCO_3$, 25 mM; $NaH_2PO_4$, 1.15 mM; D-glucose, 2.8 mM; Na pyruvate, 4.9 mM; Na fumarate, 2.7 mM; and Na glutamate, 2.7 mM; equilibrated

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[1] *Abbreviations used in this paper*: ACh, acetylcholine; $[Ca^{2+}]_i$, cytosol-ionized calcium concentration; CCK, cholecystokinin.

with 5% $CO_2$ in $O_2$ and warmed to 37°C flowed at about 10 ml/min. Measurements of cell membrane potential and resistance were done as previously described (8, 13) with two glass micro-electrodes filled with 3 M KCl (30–40 MΩ) inserted into neighboring surface cells of the same acinus under visual control (Leitz stereomicroscope, magnification ×160; E. Leitz, Inc.; Rockleigh, N. J.). A third micro-electrode filled with 2 M AChCl was placed with its tip in an extracellular position close to the impaled acinus. An indifferent Ag/$AgCl_2$ electrode was placed in the bath. Current pulses could be injected through one of the intracellular and the extracellular micro-electrodes. The details of the electrical circuits have previously been described (8, 14, 15). For the purpose of constructing dose-response curves, stimulation of the tissue was carried out by changing the fluid flowing into the bath from the normal control to one containing the stimulant in a known concentration. In other experiments in which it was desirable to demonstrate immediate and immediately reversible effects, it was more convenient to use injection of the stimulant into the bath by placing the tip of a needle connected to a syringe close to the intracellular electrodes.

*Measurement of amylase secretion.* Pancreases were divided into small segments of about 5 mg weight and a total of about 150–200 mg tissue was placed in a small translucent plastic chamber (1 ml) through which an oxygenated Krebs-Henseleit solution was pumped at a constant rate of 1 ml/min. The concentration of amylase in the effluent from the flow cell was assayed by the automated fluorometric method of Rinderknecht and Marbach (16) applied to pancreatic time-output analysis (12). This method utilizes a fluorogenic amylopectin substrate. The generation of fluorescence is a linear function of amylase concentration (12). The fluorescence output was fed into a pen recorder and thus continuous on-line records of pancreatic amylase output were obtained. α-Amylase (crystallized and lyophilized from *Bacillus subtilis*) was used as a standard for calibration. The concentration of amylase in the effluent is given in units per milliliter. The unit of amylase is defined as the amount of amylase which will liberate 1.0 mg maltose from starch in 3 min at pH 6.9 and 20°C. None of the experimental procedures reported in this paper to have effects on amylase release from the pancreas had any effect on the fluorescence intensity when there was no tissue present in the flow cell.

*Peptides, reagents, and chemicals.* Bombesin (TF/18998, Batch, N.1) was generously provided by Dr. R. de Castiglione (Farmitalia, Ricerea Chimica, Milan, Italy). Caerulein was a gift from Kyowa Hakko Kogyo, Ltd. (Tokyo, Japan). Pentagastrin was purchased from I.C.I. (U.K.). α-Amylase was type IIA from Sigma Chemical Co., St. Louis, Mo. Amylopectin anthranilate was purchased from Calbiochem, San Diego, Calif. All other chemicals were of the highest grade available.

## RESULTS

*Effect of bombesin on acinar membrane potential and resistance.* The resting acinar cell membrane potential in one series (Fig. 1) was −35.6±0.6 mV (S.E.) ($n$ = 28) and the mean input resistance was 4.4 ±0.3 MΩ ($n$ = 28). Changing the standard Krebs-Henseleit solution flowing through the bath to one containing bombesin (30 pM to 10 nM), during continuous recording from an acinus with an indwelling micro-electrode, caused a reduction in the membrane potential (depolarization) and a reduction in the membrane resistance. Dose-response curves for bombesin evoked depolarization and reduction in membrane resistance are shown in Fig. 1. 30 pM was the lowest bombesin concentration at which clear-cut depolarizations were observed. Since small spontaneous fluctuations of about 1 mV can easily occur in many cells it was impossible to obtain reliable values for the very small depolarizations that one might expect at bombesin concentrations about 10 pM.

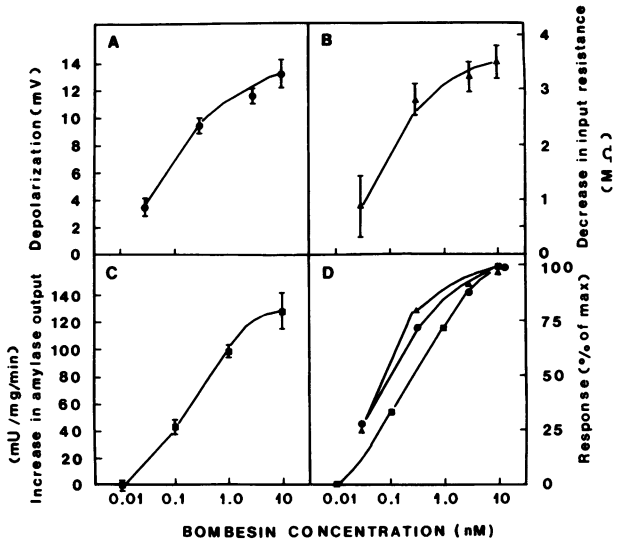


FIGURE 1 Effect of bombesin on rat pancreatic acinar cell membrane potential, membrane resistance and amylase secretion. Each point is mean±SE ($n$ = 3–11). A and B represent data from the same cells of the same glands. C represents the maximal increase in amylase output, determined from records of the type shown in Fig. 5, expressed as milliunits amylase activity per milligram pancreatic tissue (wet weight) per minute. In D the results shown in A, B, and C are plotted together to enable comparison.

In another series of experiments bombesin was administered locally into the bath in single shots by placing the tip of a fine needle connected to a syringe close to the impaled acinus. A dose of 3 pmol bombesin caused a mean depolarization of 11±1 mV and a reduction in resistance of 2.6±0.5 MΩ. These values were obtained in the presence of atropine in a concentration (1.4 μM) sufficient to block the effect of ACh (micro-iontophoretic). When two electrodes were placed in neighboring communicating cells the potential and resistance changes after bombesin stimulation were identical (Fig. 2). The time course of membrane polarizations caused by injecting rectangular current pulses was quickened considerably by the action of bombesin (not shown).

*The uncoupling effect of large doses of bombesin.* When a comparatively large dose (10 pmol) of bombesin was injected into the tissue bath or the fluid flowing through the tissue bath changed from a standard

Krebs solution to one containing bombesin (10 nM), the initial depolarization and reduction in membrane resistance (occurring simultaneously in two electrically coupled cells within an acinus) was followed by uncoupling; i.e., the current pulses injected through one of the intracellular electrodes caused a large potential change in the cell of current injection which was no longer transmitted to the neighboring cell (Fig. 2). This uncoupling action was fully reversible. Similar uncoupling effects were obtained after large doses of ACh (Fig. 2), pentagastrin, and caerulein (not shown).

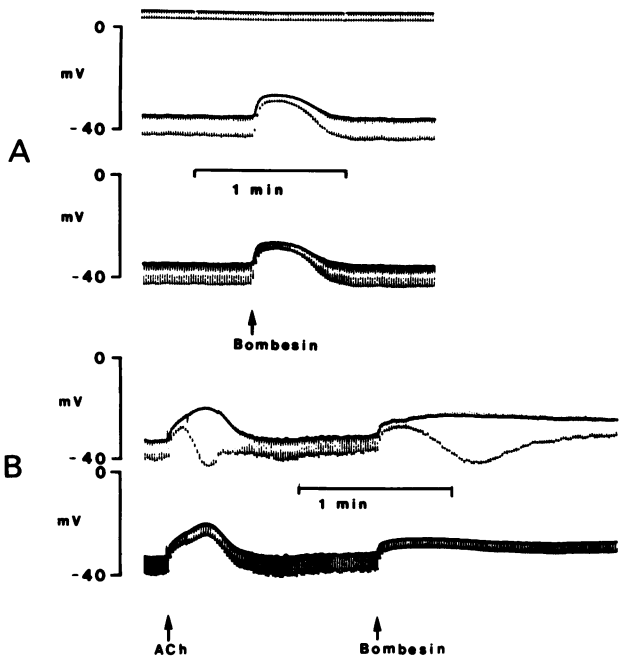


FIGURE 2 Effect of bombesin on mouse acinar cell membrane potential, resistance, electrical time constant, and electrical coupling. A: Pen recordings showing simultaneous measurement of membrane potentials from two communicating acinar cells. Top trace represents time base. Short pulses at second, long pulses at minute intervals. Rectangular, constant size current pulses (100 ms, 2 nA) (not shown) were repetitively injected through the micro-electrode recording the potential represented by the middle tracing, this caused shortlasting membrane hyperpolarizations in the same cell (middle trace) and also in the neighboring cell (bottom trace). At the time corresponding to the arrow marked bombesin, 3 pmol of the tetradecapeptide was injected into the tissue bath. Note that the two cells remain tightly coupled throughout the trace (the coupling ratio, i.e., current pulse-induced potential change in cell of current injection divided by electrotonic potential change in neighboring cell, is 1). B: Pen recordings showing simultaneous measurement of membrane potential from two communicating acinar cells. Rectangular current pulses of constant size (100 ms, 2.5 nA) (not shown) injected through micro-electrode recording potential represented by upper trace. At arrow marked ACh an ejecting current pulse (80 nA, 10 s) was applied to the extracellular AChCl-containing micro-electrode (retaining current: 20 nA). At arrow marked bombesin, 10 pmol of the peptide was injected into the bath. Note that the two cells are tightly coupled before stimulation, but that after the initial stimulus-evoked membrane resistance reduction there is a transient large increase in current-pulse evoked hyperpolarizations in the cell of current injection (upper trace) which are only partially transmitted to the neighboring cell (below), this is a sign of electrical uncoupling.

*The bombesin equilibrium potential.* Stimulating with bombesin during repetitive injections of large depolarizing current pulses into one cell and recording the membrane potential from the neighboring cell with the other micro-electrode, it was possible to assess simultaneously the effect of bombesin at two different levels of membrane potential; the spontaneous resting potential and an artificial low potential caused by the current passage. Fig. 3 shows an example of such a record in which a comparison between the effects of bombesin, ACh, pentagastrin, and caerulein is made. At the spontaneous resting potential of −30 mV all stimulants caused depolarization, whereas at the lowered resting potential of about −7 mV all stimulants caused hyperpolarization. From records such as the one shown in Fig. 3 it is possible to estimate the membrane potential at which a stimulant would cause no potential change (equilibrium or null potential). The bombesin equilibrium potential assessed in this way was −15.6±1.3 mV ($n$ = 4). The ACh equilibrium potential measured in the same cells was −16.1±0.7 mV ($n$ = 3). The caerulein equilibrium potential was −16 mV ($n$ = 2) and the pentagastrin equilibrium potential was −16 mV ($n$ = 2).

*Effect of bombesin on amylase secretion.* Immediately after placing the pancreatic segments in the flow chamber there was a very large release of amylase in the absence of hormonal stimulation. During the 1st 0.5–1.0 h the amylase output gradually declined and after 1 h of superfusion with Krebs-Henseleit solution a constant amylase output (about 10 mU/min per mg) was attained. Bombesin (0.1 to 10 nM) caused, after a latency of 1.2±0.2 min ($n$ = 12), a sharp rise in amylase output very similar to that seen after ACh stimulation (Fig. 4). The amylase output peaked after 5 min and then declined in spite of sustained stimulation to reach a much lower stable release rate. The rise in fluorescence intensity after 10 nM bombesin stimulation was as steep as it could possibly be with the measuring system used (compare with steepness of amylase calibration curve in Fig. 4). The dose-response curve for the bombesin-evoked maximal secretion is shown in Fig. 1.

## DISCUSSION

The effect of bombesin on pancreatic acinar cell potential and resistance described in this paper is remarkably similar (also quantitatively) to the effects of ACh, cholecystokinin (CCK), caerulein, and gastrin

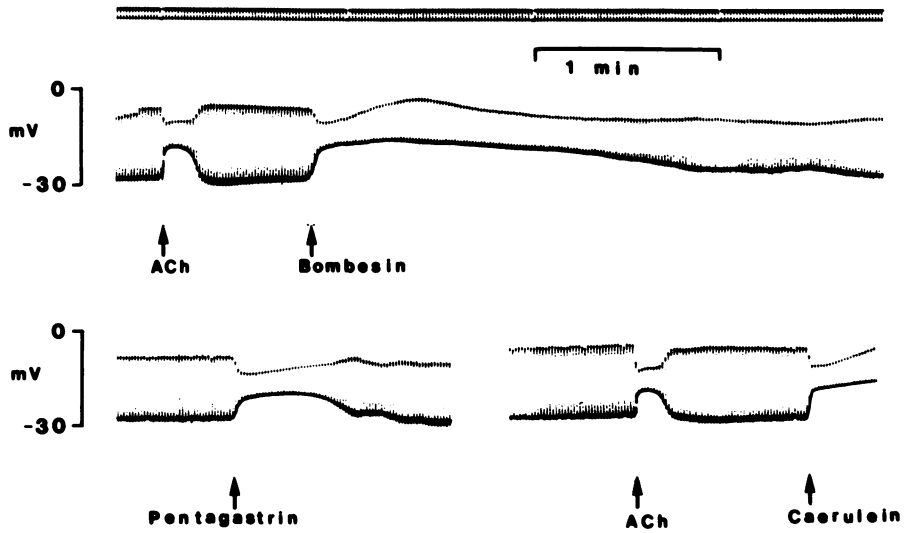


FIGURE 3 The assessment of ACh-, bombesin-, pentagastrin- and caerulein-equilibrium potential in rat pancreas. Three consecutive records from the same acinus. The heavy trace represents the spontaneous membrane potential. The interrupted trace (above) represents the membrane potential during the repetitive injections of depolarizing rectangular current pulses (5 nA, 100 ms) (not shown) through a micro-electrode inserted into a communicating neighboring cell. The effects of the stimulants at two different levels of membrane potential (−30 mV and −7 mV) are therefore obtained. At the artificial low potential all stimulants induce hyperpolarization, whereas at the spontaneous resting potential the usual depolarizations are seen. In this case the null or equilibrium potential is within the range −12 to −18 mV and for the purpose of calculating mean values of the equilibrium potential a value of ½ (−12 + [−18]) = −15 mV would have been assigned to this particular cell. ACh was applied by micro-iontophoresis (60 nA ejecting current for 0.5 s, retaining current: 20 nA), bombesin, pentagastrin, and caerulein were given as single shot injections to the bath in doses of 3 pmol, 3.5 nmol, and 1.5 pmol, respectively.

described previously in the rat and mouse (7–11, 13, 17). The effects of bombesin in rat and mouse were similar (Figs. 2 and 3), in agreement with previous data showing similar actions of ACh in rat and mouse pancreatic acinar cells (8). The simultaneously occurring, bombesin-evoked identical resistance reduction measured in two neighboring electrically communicating acinar cells within an

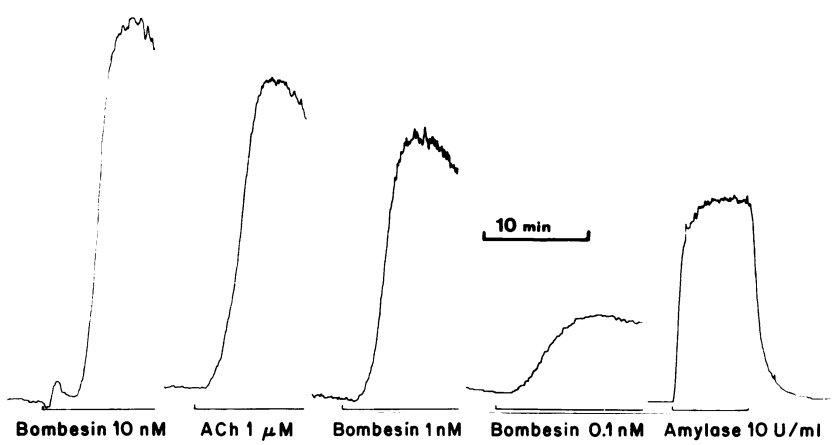


FIGURE 4 Original fluorescence tracings from effluent of pancreas superfusion chamber. Each trace is from a separate experiment (rat) the stimulant being added to the superfusion fluid in the concentration stated 1 h after setting up the preparation. To the right is shown a calibration curve obtained by adding α-amylase to the Krebs-Henseleit solution flowing through the system at the concentration shown.

acinus (Fig. 2 upper part) is direct proof that bombesin, like ACh (13), reduces the surface cell membrane resistance. The complete congruity between the dose-response curves for bombesin-evoked depolarization and resistance reduction was expected since there is a rigid relationship between transmitter-evoked potential and resistance changes (13, 18).

The relationship between the stimulant-evoked electrical membrane changes and secretion are not yet clear. The dose-response curves for bombesin-evoked resistance change, depolarization, and amylase secretion are very similar as they are in the case of ACh (19). Depolarization of the pancreatic acinar cell membrane by excess extracellular K does not evoke amylase secretion if atropine is present to prevent the action of ACh liberated from depolarized nerve endings (20, 21). This finding does not, however, indicate that the ACh, CCK, or bombesin-evoked membrane changes are unimportant. High extracellular K depolarizes the acinar cell membrane without changing the membrane resistance (8) whereas ACh, CCK, gastrin, pentagastrin, caerulein, and bombesin all evoke a marked reduction in acinar cell surface membrane resistance (8–10). Stimulating pancreatic amylase secretion by raising the cytosol-ionized calcium concentration without receptor activation, with the divalent cation ionophore A23187 or direct intracellular $Ca^{2+}$ injection, also causes membrane resistance reduction and depolarization (21, 22).

The values of the equilibrium potential for the action of bombesin, ACh, pentagastrin, and caerulein were similar and also similar to the previously directly determined value for the ACh equilibrium potential (15). The peptides clearly act on receptors independent of the cholinergic receptors, since their action is unimpaired by the presence of atropine (3, 7, 9). Bombesin probably does not act on the same receptor sites as CCK, caerulein, or gastrin since it is structurally very different and does not, in contrast to CCK, stimulate adenyl cyclase in pancreatic plasma membrane preparations (4). If the excitation of the three different pancreatic acinar receptor sites had resulted in three different values for equilibrium potential, then we would have been able to conclude that the mechanisms of action of the three groups of stimulants were different. The experimental result showing that the equilibrium potentials are identical suggests, but does not finally prove, that the mechanisms of action are the same. ACh, CCK, and bombesin evoke quantitatively identical membrane responses with respect to maximum depolarization, resistance reduction and equilibrium potential, they all evoke an increase in $^{45}$calcium outflux from prelabeled glands (3, 19, 23–28), evoke the same maximal amylase secretion, have similar shaped dose-response curves for amylase secretion, increase in Ca outflux, and membrane depolarization (Fig. 1) (3, 7, 19, 26), and have similar dynamic profiles of amylase output following stimulation (Fig. 4). These findings strongly suggest that all three groups of stimulants (activating ACh, CCK, and bombesin receptors) have identical mechanisms of action.

The finding that bombesin, ACh, and caerulein can all, employing relatively high doses, evoke electrical uncoupling of acinar cells within an acinus (Fig. 2), as already shown for ACh stimulation alone (22), is the most direct demonstration presently available of an increase in cytosol-ionized calcium concentration ($[Ca^{2+}]_i$) caused by stimulation. It is extensively and directly documented that the conductance of the junctional channels through which intercellular electrical communication occurs is controlled by $[Ca^{2+}]_i$ in such a way that the permeability is drastically reduced above a certain level of $[Ca^{2+}]_i$ (29, 30). Intracellular $Ca^{2+}$ injection to pancreatic acinar cells causes electrical uncoupling (22).

The surface cell membrane conductance change after ACh stimulation in pancreatic acini is mainly due to an increase in Cl, Na, and K conductance causing an influx of Na and Cl and an outflux of K (15). The quantitative similarity of the action of bombesin on the acinar cell electrophysiological properties to that of ACh therefore suggests that bombesin also causes similar transmembrane ion fluxes. The recent findings that the calcium ionophore A23187 and intracellular Ca application by micro-iontophoresis to pancreatic acinar cells induce membrane depolarization and resistance reduction (21, 22) suggest that the plasma membrane Cl, Na, and K conductance changes evoked by ACh, bombesin, and CCK may be mediated by an increase in $[Ca^{2+}]_i$. This would explain why the equilibrium potentials for the action of all three groups of agonists acting on three different receptor sites are identical (Fig. 3), and is in agreement with the finding that all these stimulants can evoke electrical uncoupling of acinar cells.

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