Bombesin-Induced Changes in Membrane Potential-Dependent Phasic Contractions of Cat Gastric Muscle

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Abstract. Electrical and contractile activities of smooth muscle strips isolated from the circular muscle layer of cat gastric antrum were studied using the sucrose gap technique. Bombesin (10^{-8} mol/l) depolarized the gastric muscle; this was accompanied by an increase in the strip tone, in the plateau action potential frequency and in both the frequency and the amplitude of the spike potentials as well as by a shortening of the plateau action potential duration. Both the frequency and the amplitude of the phasic contractions increased thereafter. The changes in the frequency of the plateau action potentials and contractions were not influenced either by antagonists of cholinergic and adrenergic receptors or by TTX. In the presence of the Ca antagonists D600 (10^{-6} mol/l) and nifedipine (10^{-7} mol/l) or in Ca-free medium containing EGTA the effect of bombesin on the frequency of the plateau action potentials and phasic contractions remained unchanged; however, spike potentials were not observed and no increase in the amplitude of phasic contractions occurred. UV-light inactivation of nifedipine restored the typical bombesin effect on the electrical and contractile activities of the gastric smooth muscle. The present data suggest that the effect of bombesin on the frequency of both plateau action potentials and phasic contractions is not linked with Ca^{2+} influx.

Key words: Gastric smooth muscle — Contractions — Potentials — Bombesin

Introduction

Bombesin is known to increase the frequency of slow potentials generated in vivo by the canine gastric smooth muscle; it leads to the appearance of spike potentials or increases both the amplitude and the frequency of the existing spike activity (Caprilli et al. 1975), and enhances gastric motility (Klimov et al. 1985). An increase in the amplitude and frequency of the phasic contractions has

also been observed in in vitro experiments. The effects of bombesin on circular muscle strips were not influenced by TTX while on longitudinal muscle strips the bombesin effects were blocked by TTX (Mayer et al. 1982). Establishing a strong excitatory effect of bombesin in all regions of the human stomach and only in the corpus of the canine stomach, Lüdtke et al. (1984) suggested species specificity of the bombesin excitatory effects. These effects may be realized: (i) through bombesin-modulated release of a neurotransmitter via presynaptic receptors localized on the intramural nerves (Fox et al. 1982; Mayer et al. 1982; Fox and McDonald 1984); or (ii) through direct stimulation of the smooth muscle cells (Bertaccini and Impicciatore 1975; Mukhopadhyay and Kunnemann 1979; Girard et al. 1984). The fact that bombesin induced changes in gastric electrical activity under in vivo conditions stimulated our interest in studying the effects of the peptide on the spontaneous activity of the cat gastric antral muscle in vitro.

Materials and Methods

The stomachs of chloralose (80 mg/kg) anesthetized cats were removed and placed in Krebs solution. Mucosa-free muscle strips (2 × 20 mm) were cut from the circular layer of the antrum. The electrical and contractile activities were measured using the sucrose gap technique (Boev and Golenhofen 1974). Modified Krebs solution bubbled with 95% O_2 and 5% CO_2 was used and experiments were performed at 36°C.

In a series of experiments intended to evaluate the importance of extracellular Ca^{2+} for the realization of the bombesin effects, we employed UV-light, which is known to rapidly inactivate the Ca-antagonist nifedipine and to restore the contractile activity of heart and smooth muscles (Morad et al. 1983; Boev et al. 1986). To prevent the occurrence of desensitization each administration of bombesin was followed by washouts of the strips with Krebs solution for one hour.

Solutions and substances used. The composition of the Krebs solution used was (mmol/l): Na^+ 137.5; K^+ 5.96; Mg^{2+} 1.2; Ca^{2+} 2.5; Cl^- 124.6; $H_2PO_4^-$ 1.19; HCO_3^- 2.5; glucose 11.5. Drugs used: phentolamine (Hoffman—La Roche), propranolol (Fluka), atropine (Pharmachim), tetrodotoxin (TTX) (Sankyo), methoxyverapamil (D600) (Knoll), nifedipine (Bayer), bombesin (All-Union Research Center of Cardiology, Moscow), EGTA (Sigma).

Results

Smooth muscle strips isolated from the gastric antrum were found to contract spontaneously with a frequency of 1.5—2 cpm. Bombesin at a threshold concentration of 10^{-14} mol/l increased in a dose-dependent manner the contraction amplitude and maximal effect was obtained at 10^{-8} mol/l. The effect weakened at concentrations exceeding 10^{-8} mol/l.

Effect of bombesin on gastric electrical and contractile activities. The cat gastric antral muscle is characterized by generation of spontaneous rhythmic plateau

action potentials with a complex configuration: an initial fast component followed by a second relatively slow component. In some preparations spike potentials were superimposed on the second component. These changes in the membrane potential occurred at a frequency of 1.5 to 2 cpm. They were accompanied with phasic contractions of the same frequency. Bombesin at the concentration used (10^{-8} mol/l) induced changes in the electrical and contractile activities of the gastric muscle accompanied by a pronounced depolarization (Fig. 1). A slight increase in the tone was also observed in some preparations.

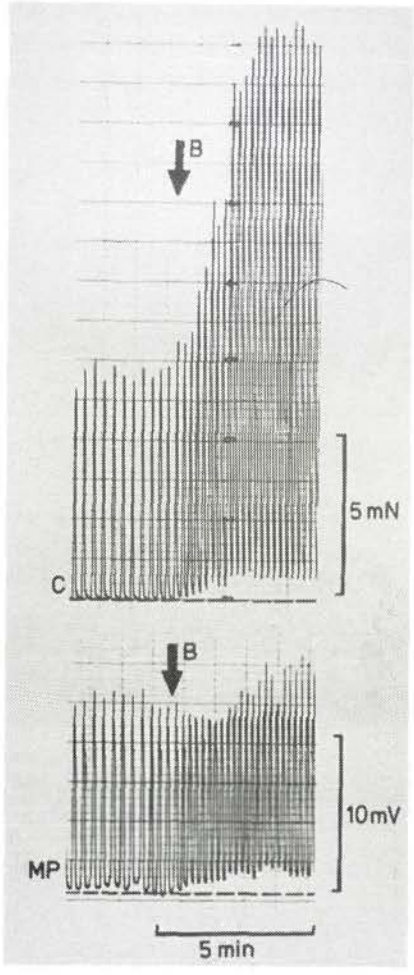

Fig. 1. Bombesin-induced increase in the frequency of the plateau action potentials and in the amplitude and frequency of the spike potentials and phasic contractions. *Lower trace:* electrical activity; *upper trace:* contractions.

Fig. 1 shows that the background frequency of the plateau action potential (2 cpm) increased to 4 cpm during the first min after bombesin addition and reached its maximum (6 cpm) during the third min. This was accompanied by

a shortening of the duration of the plateau action potential and by an increase in the spike potential amplitude. However, the amplitude of the first potential component was not altered. These changes correlated with the shortening of the duration of the plateau action potential-related phasic contractions, and their amplitude was also increased. Bombesin induced synchronous rhythmic activity even when (in controls) the plateau action potentials and phasic contractions were not rhythmic. It is evident from Fig. 1, that the amplitude of the plateau action potential did not significantly change and that the increase in the amplitude of the phasic contraction resulted mainly from an increase in both the amplitude and the frequency of the spike potentials. These changes persisted over 20—30 min after the washout of bombesin. The effect of bombesin on both the frequency of the plateau action potentials and phasic contractions was observed after the addition of antagonists of cholinergic and adrenergic receptors as well as after TTX (Fig. 2).

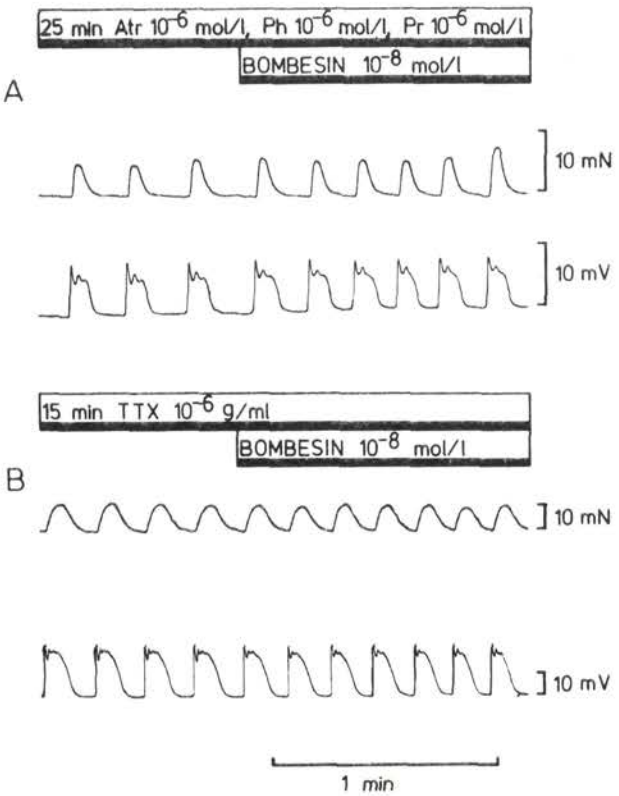

Fig. 2. Effect of bombesin following treatment with antagonists of cholinergic and adrenergic receptors (*A*) or TTX (*B*). The same arrangement as in Fig. 1.

Extracellular Ca^{2+} and the effect of bombesin. The Ca antagonist D600 reduced the second component of the plateau action potentials and greatly decreased

both the frequency and the amplitude of the phasic contractions. On this background bombesin produced depolarization and increased the frequency of both the plateau action potentials and the related phasic contractions (Fig. 3).

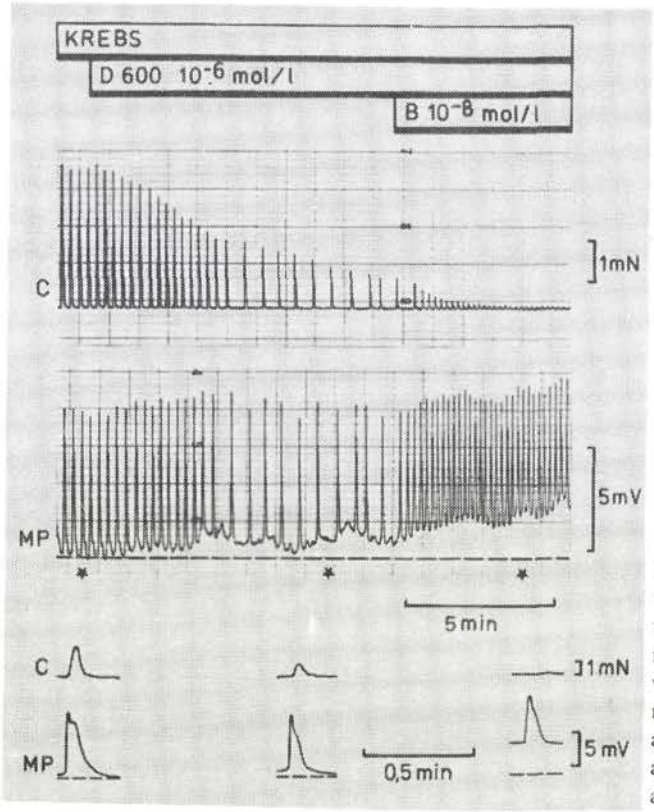

Fig. 3. Effect of bombesin on the electrical and contractile activities of the gastric smooth muscle following treatment with D600. The same arrangement as in Fig. 1. Contractions and electrical activity recorded at high paper speed are shown at the bottom.

The spike potential-inducing mechanism, characteristic of bombesin, was not manifested. Nifedipine, a well-known Ca antagonist, when administered on the background of spontaneous spike activity, inhibited the generation of spike potentials, and reduced both the duration and the amplitude of the second component of the plateau action potentials as well as the related phasic contractions. Upon UV-irradiation of the strips the duration of the plateau action potential recovered, together with the spike activity and the amplitude of the phasic contraction (Fig. 4*A*). In the presence of nifedipine bombesin only exerted its characteristic effect on the frequency of both the plateau action potentials and the phasic contractions: it increased the frequency and decreased the duration of the plateau action potentials (Fig. 4*B*). On this background UV-light led to the appearance of spike activity and to a significant increase in

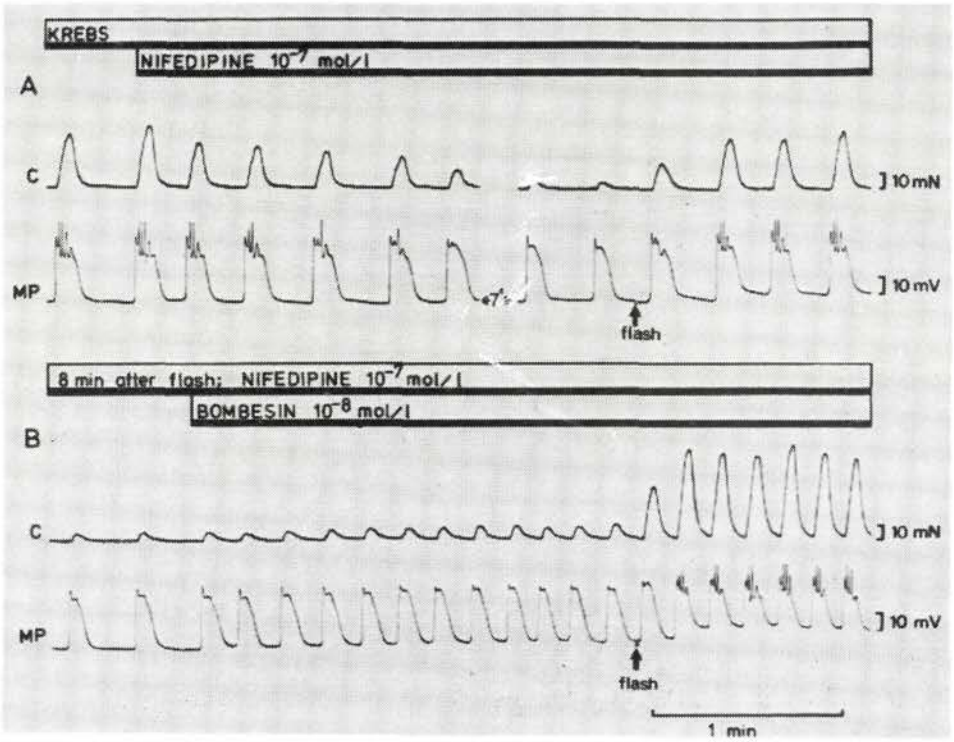

Fig. 4. Effect of bombesin on the electrical and contractile activities of the gastric smooth muscle after treatment with nifedipine and following UV-light inactivation of nifedipine. (*A*) control; (*B*) after UV-light. The same arrangement as in Fig. 1.

the amplitude of phasic contractions (cf. Fig. 4*A* and Fig. 4*B*), i.e. after light-induced inactivation of nifedipine the typical effect of bombesin on spike activity and phasic contractions was restored. Moreover, UV-light pulses led to a further depolarization followed by an increase in the strip tone. Perfusion of the strips with Ca-free solution containing 0.5 mol/l EGTA for 15—20 min greatly reduced both the amplitude and the frequency of the plateau action potentials and inhibited the spike potentials and spontaneous contractions. On this background bombesin increased the frequency of both the plateau action potentials and phasic contractions as well as the amplitude of the phasic contraction. However, no spike activity occurred (Fig. 5). The addition of Ca^{2+} to the solution restored the effect of bombesin on the electrical and contractile activities.

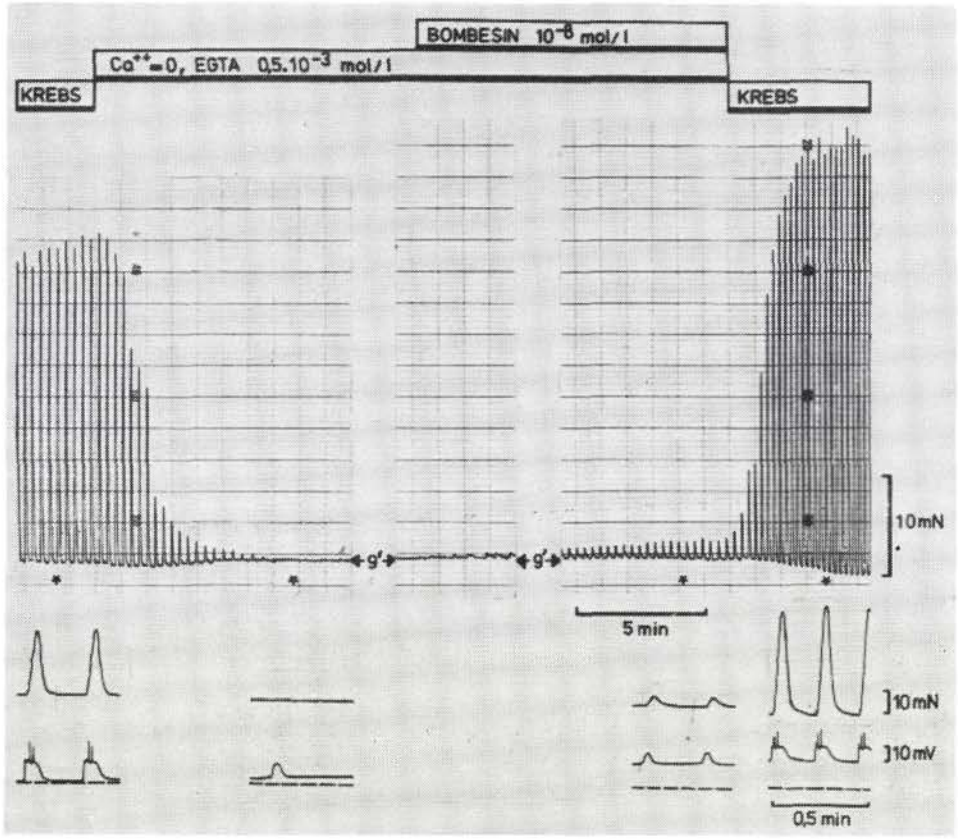

Fig. 5. Effect of bombesin added to Ca^{2+}-free medium on gastric smooth muscle contractions. The same arrangement as in Fig. 3.

Discussion

Contractions of the smooth muscle are determined by the Ca^{2+} level in the cytoplasm. Sources of calcium are extracellular Ca^{2+} and Ca^{2+} released from the intracellular stores (Casteels et al. 1972; Brading and Sneddon 1980). It is known that phasic contractions of the gastric smooth muscle are related both to Ca^{2+} influx through the cell membrane and to Ca^{2+} release from the stores, and that extracellular Ca^{2+} determines the spontaneous tone (Boev 1978). There is also evidence that phasic contractions of the smooth muscle of canine, cat and human stomach are triggered by plateau action potentials (Papasova et al. 1968; Papasova et al. 1972; Papasova and Boev 1976).

The patterns of both the plateau action potentials and the related phasic

contractions are modulated by different neurotransmitters and biologically active peptides (Szurszewski 1981; Sanders and Szurszewski 1981). The present observations show that bombesin increases the frequency of both the plateau action potentials and the phasic contractions in cat gastric muscle. Changes in the frequency of the plateau action potential have also been observed in conscious dogs on intravenous injection or infusion of the peptide (Caprilli et al. 1975). The fact that the bombesin-induced changes in the plateau action potentials are not influenced by antagonists of adrenergic and cholinergic receptors or by TTX suggests that they are not associated with any neurotransmitter release. This suggestion is in accordance with the report of Mayer et al. (1982) that in circular muscle strips from canine stomach bombesin-induced changes in spontaneous contractions were TTX-insensitive as contrasted with TTX-sensitive changes in longitudinal strips. Changes in the frequency of both the plateau action potentials and the related phasic contractions were also observed in Ca^{2+}-free medium containing EGTA as well as after the Ca antagonists D600 and nifedipine. Mayer et al. (1982) have also reported that the bombesin-induced changes in the frequency of both the plateau action potential and the contractions of gastric smooth muscle are not influenced by verapamil. All this evidence taken together suggests that the effects of bombesin on the frequency of both the plateau action potentials and the related contractions are not associated with Ca^{2+} influx.

The present results also show that bombesin induces the appearance of spike potentials and an increase in their amplitude and frequency when administered on the background of spontaneous spike activity. This was accompanied by marked depolarization, which was occasionally associated with an increase in the strip tone (Fig. 1). There is also evidence that the generation of spike potentials in the smooth muscle, including the gastric muscle, is related to Ca^{2+} influx through the cell membrane (Papasova et al. 1968; Weigel et al. 1979), which is thought to result from the activation of membrane potential-dependent Ca^{2+} channels (Bolton 1979). Probably, the bombesin effect on the spike activity is realized by an increase in Ca^{2+} influx through the membrane potential-dependent Ca^{2+} channels. This assumption is supported by the fact that in the presence of the Ca^{2+} antagonists D600 and nifedipine bombesin failed to evoke spike potentials although an increase in the frequency of both the plateau action potentials and the phasic contractions was observed.

In conclusion, it might be suggested that bombesin is able to stimulate the spontaneous electrical activity of gastric smooth muscle and the related phasic contractions: (i) through influencing membrane potential-dependent Ca^{2+} channels; this results in the occurrence or increase of spike activity; and (ii) through releasing Ca^{2+} from the intracellular stores by a still unknown mechanism; this results in an increase in both the frequency of the plateau action

potentials and the frequency and amplitude of the phasic contractions. The fact that in Ca^{2+}-free medium containing EGTA bombesin increases the frequency of both the plateau action potentials and the phasic contractions as well as the amplitude of the latter strongly suggests that Ca^{2+} released from the intracellular stores might be responsible for the excitation-contraction coupling in gastric smooth muscle.

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