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Role of prostaglandin E_2 and Ca^{2+} in bradykinin induced contractions of guinea-pig gallbladder in vitro

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Abstract

In this study, we investigated the contribution of prostaglandin E_2 to bradykinin induced contractions of guinea-pig gallbladder in vitro and characterized the sources of activator Ca^{2+} for the bradykinin mediated contractions. Contractions induced by bradykinin in guinea-pig gallbladder smooth muscle strips were significantly attenuated by the cyclooxygenase inhibitor piroxicam (10 μM). In the presence of piroxicam, a threshold concentration of prostaglandin E_2 (1 nM) significantly enhanced the contractile response to subsequent challenge with bradykinin. Contractile responses to bradykinin were abolished in a Ca^{2+}-free medium plus EDTA. The inhibitor of receptor mediated Ca^{2+} entry, SK&F 96365 (1-[β-[3-(4-methoxyphenyl)-propoxy]-4-methoxyphenethyl]-1*H*-imidazole hydrochloride, 10–50 μM) dose dependently abolished the response to bradykinin, while this response was only partially attenuated by nifedipine (10–50 μM; a voltage-operated Ca^{2+} channel antagonist). Thapsigargin (an inhibitor of the sarcoplasmic reticulum calcium ATP-ase pump, 1 μM) produced sustained contractions of guinea-pig gallbladder strips that were dependent on extracellular Ca^{2+}. After incubation of strips in a Ca^{2+}-free medium with thapsigargin, replacement of Ca^{2+} caused a large sustained contraction. We conclude that the contractile response of guinea-pig gallbladder to bradykinin is modulated by prostaglandin E_2. Bradykinin induced contractions of guinea-pig gallbladder are highly dependent on extracellular Ca^{2+} which enters through store-operated Ca^{2+} channels and partially through voltage-operated Ca^{2+} channels.

Keywords: Gallbladder; Bradykinin; Prostaglandin E_2; Ca^{2+} channel

1. Introduction

Bradykinin is a biologically active nonapeptide, which is a potent mediator of inflammation, smooth muscle contraction and nociception. Bradykinin is formed in tissue and plasma by the action of kallikreins on kininogens (Bhoola et al., 1992). Two bradykinin receptors have been identified; B_1 and B_2 (Regoli et al., 1998). The bradykinin B_2 receptor is present in most tissues while the bradykinin B_1 receptor is typically expressed de novo after inflammatory stimuli or tissue injury (Marceu et al., 1998). Bradykinin has been shown to be capable of stimulating prostaglandin release in a variety of tissues including the gallbladder (Myers et al., 1992; Bogar et al., 1999). Kinins (German et al., 1989), as well as prostanoids (LaMorte et al., 1985; Moser et al., 2000), have been reported to contribute to the pathogenesis of gallbladder disease. Moreover, studies regarding the potential contribution of kinins to gallbadder disease implicate local prostanoid formation (German et al., 1989; Myers et al., 1993; Bogar et al., 1999).

In the guinea-pig gallbladder, bradykinin has been shown to evoke smooth muscle contraction by activation of B_2 receptors (Falcone et al., 1993; Cabrini et al., 1995) as well as stimulating bicarbonate secretion across the epithelium (Baird and Margolius, 1989). These bradykinin related actions upon gallbladder tissues appear to be dependent on prostanoid formation since they can be attenuated by cyclooxygenase inhibitors (Baird and Margolius, 1989; Falcone et al., 1993; Cabrini et al., 1995). However, it is not clear whether prostaglandins directly mediate the effects of bradykinin in the gallbladder or whether bradykinin is having a direct effect on the tissue itself with prostaglandins acting to potentiate or modulate bradykinin action.

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Additionally, conflicting observations have been presented regarding the sources of activator Ca^{2+} for B_2 receptor mediated contraction in the guinea-pig gallbladder. (Cabrini and Calixto, 1997; Cabrini et al., 1995). Smooth muscle contraction is dependent on an increase in the concentration of free cytosolic Ca^{2+}, which may result from influx of extracellular Ca^{2+} through ion channels on the plasma membrane and/or by release of Ca^{2+} from the sarcoplasmic reticulum. Influx of extracellular Ca^{2+} can occur through at least three types of ion channels, voltage-operated Ca^{2+} channels, receptor-operated Ca^{2+} channels (Barritt, 1999; Hofman et al., 1999) and store-operated Ca^{2+} channels (Berridge, 1995). Information in this field would be of particular interest because of the possible use of drugs with Ca^{2+} entry blocking properties in the management of biliary colic.

The current study was undertaken to examine the role of prostaglandins in bradykinin induced guinea-pig gallbladder contractions and to investigate the sources of activator Ca^{2+} for bradykinin induced contractions. We chose to perform these studies with no exogenous peptidase inhibitor present. Although contractile responses of guinea pig isolated gallbladder to bradykinin (Woods and Baird, 1992) and tachykinins (Maggi et al., 1989) have been shown to be enhanced in the presence of peptidase inhibitors, the profile of matched responses is not altered. In the absence of clinical reports linking captopril administration with increased incidence of gallbladder disease, it may be that the influence of peptidase inhibitors in vitro may have more research implications than therapeutic significance (Thrall and Swanson, 1989).

2. Materials and methods

2.1. Tissue preparation

Adult guinea-pigs were killed by cervical dislocation and the gallbladder was removed. The gallbladder was opened and washed several times in Krebs solution to remove bile. Usually, four strips of each gallbladder approximately 0.5 cm wide $\times$ 1.5 cm long were mounted in 15 ml organ baths containing Krebs solution of the following composition (mM): NaCl, 118; KCl, 4.7; $MgSO_4$, 1.2; KH_2PO_4, 1.2; glucose, 11.1; $NaHCO_3$, 24.9 and $CaCl_2$, 2.5, maintained at 37 °C and gassed with 95% O_2 and 5% CO_2. A resting pre-load of 0.5 g was applied to each muscle strip which was then allowed to equilibrate for 1 h, during which time the Krebs solution was changed every 20 min. Tissue strips were then exposed to carbachol (10 μM) in order to confirm the viability of the tissues and to determine the maximum contractile capacity. The carbachol was then washed out and the preparations were left to re-equilibrate for approximately 30 min before drug addition. Tissues eliciting less than 1 g of tension in response to carbachol were discarded. Mechanical activity was recorded using isometric transducers (World Precision Instruments, Stevenage, Herts, UK). Tension was continuously monitored and recorded using a MacLab data acquisition system (AD Instruments, Hastings, UK). In all experiments performed, separate control and test tissues were studied simultaneously in order to correct for any time dependent changes in responsiveness to bradykinin.

2.2. Effect of prostanoids on bradykinin-induced contraction

To assess the contribution of prostanoids to the contractile response elicited by bradykinin, following the equilibration period, cumulative concentration–response curves to bradykinin (0.1 nM–10 μM) were obtained in the absence and presence of piroxicam (a cyclooxygenase inhibitor, 10 μM) added 20 min before the addition of bradykinin. In separate experiments, cumulative concentration–response curves to prostaglandin E_2 (0.1 nM–1 μM) were performed in the presence and absence of piroxicam (10 μM). The effect of a threshold concentration of prostaglandin E_2 (1 nM) in the presence of piroxicam (10 μM) on the contractile response to bradykinin was then assessed. The concentration of prostaglandin E_2 was selected as that inducing less that 5% of the maximal response to carbachol. Tissues were exposed to prostaglandin E_2 for 5 min following incubation with piroxicam for 15 min before cumulative concentration–response curves to bradykinin were performed.

2.3. Effect of bradykinin on prostaglandin E_2 release

Small samples of guinea-pig gallbladder tissue were placed in separate wells of a twelve well plate containing 2 ml oxygenated Krebs solution at 37 °C. Samples were washed twice over a 10-min period before commencing stimulation studies. Treatments were as follows: control; piroxicam (10 μM); bradykinin (10 μM) and bradykinin (10 μM) plus piroxicam (10 μM). Bradykinin contact time was 5 min. The total collection time was 15 min for all experiments. Supernatants were stored at −70 °C and later analysed for prostaglandin E_2 content using a commercial enzyme immunoassay kit according to the manufacturer's instructions. The gallbladder tissues were dried and weighed. Results are expressed as picograms of prostaglandin E_2 released per milligram tissue.

2.4. Role of Ca^{2+} in bradykinin-induced contraction

To assess the contribution of extracellular Ca^{2+} to the bradykinin-induced contraction of guinea-pig gallbladder, cumulative concentration–response curves to bradykinin (0.1 nM–10 μM) were performed in normal Krebs solu-

tion and in a Ca^{2+}-free Krebs solution containing EDTA (1 mM). Tissues were maintained for 20 min in the Ca^{2+}-free medium plus EDTA, during which time the bath solution was changed twice before responses to bradykinin were obtained. In a separate set of experiments, cumulative concentration–response curves were obtained for bradykinin in the presence and absence of (i) nifedipine (L-type voltage-operated Ca^{2+} channel blocker, 10 μM–50 μM) or (ii) SK&F 96365 (1-[β-[3-(4-methoxyphenyl)-propoxy]-4-methoxyphenethyl]-1*H*-imidazole hydrochloride, 10 μM–50 μM), an inhibitor of receptor-mediated Ca^{2+} entry including store-operated Ca^{2+} channels (Merritt et al., 1990; Jan et al., 1999). Contact time for each antagonist was 20 min. To assess the possible involvement of extracellular Ca^{2+} influx through store-operated Ca^{2+} channels in excitation–contraction coupling in the guinea-pig gallbladder, tissues were treated with thapsigargin (an inhibitor of the sarcoplasmic reticulum calcium ATP-ase pump which depletes sarcoplasmic stores of Ca^{2+}) in normal Krebs and in a Ca^{2+}-free Krebs solution. Following treatment with thapsigargin (1 μM) for 30 min in the Ca^{2+}-free medium, Ca^{2+} (2.5 mM) was added.

2.5. Statistical analysis

Data are presented as mean ± S.E.M. Contractile responses to bradykinin and prostaglandin E_2 are expressed as a percentage of the maximum carbachol response. Statistical analysis of the data was performed using the paired or unpaired Student's *t*-test, or analysis of variance, when applicable. *P* values of less than 0.05 were considered to be significant.

2.6. Materials

Carbachol, bradykinin, nifedipine, prostaglandin E_2, piroxicam, thapsigargin and dimethylsulphoxide were all obtained from Sigma-Aldrich Ireland, Dublin 24, Ireland. SK&F 96365 was purchased from CN Biosciences UK, Nottingham, NG9 2JR, England. The prostaglandin E_2 enzyme immunoassay kit was obtained from Amersham Pharmacia Biotech UK, Buckinghamshire, England. Nifedipine, thapsigargin and piroxicam were made up in dimethyl sulphoxide. Carbachol and SK&F 96365 were dissolved in water. Stock solutions (10 mM) of bradykinin and prostaglandin E_2 were made up in 5% acetic acid and 95% ethanol, respectively, and diluted in Krebs solution. Stock solutions of carbachol, bradykinin, prostaglandin E_2 and thapsigargin were kept in siliconized plastic tubes, maintained at −18 °C. A stock solution of SK&F 96365 was prepared and maintained at room temperature. Piroxicam was made up immediately before use. The experiments with nifedipine were protected from light to avoid its photo-degradation. The final bath concentration of dimethyl sulphoxide did not exceed 0.1%. It had no effect on the basal tone of the preparations or on bradykinin or prostaglandin E_2 mediated contractions. Vehicle controls were used throughout.

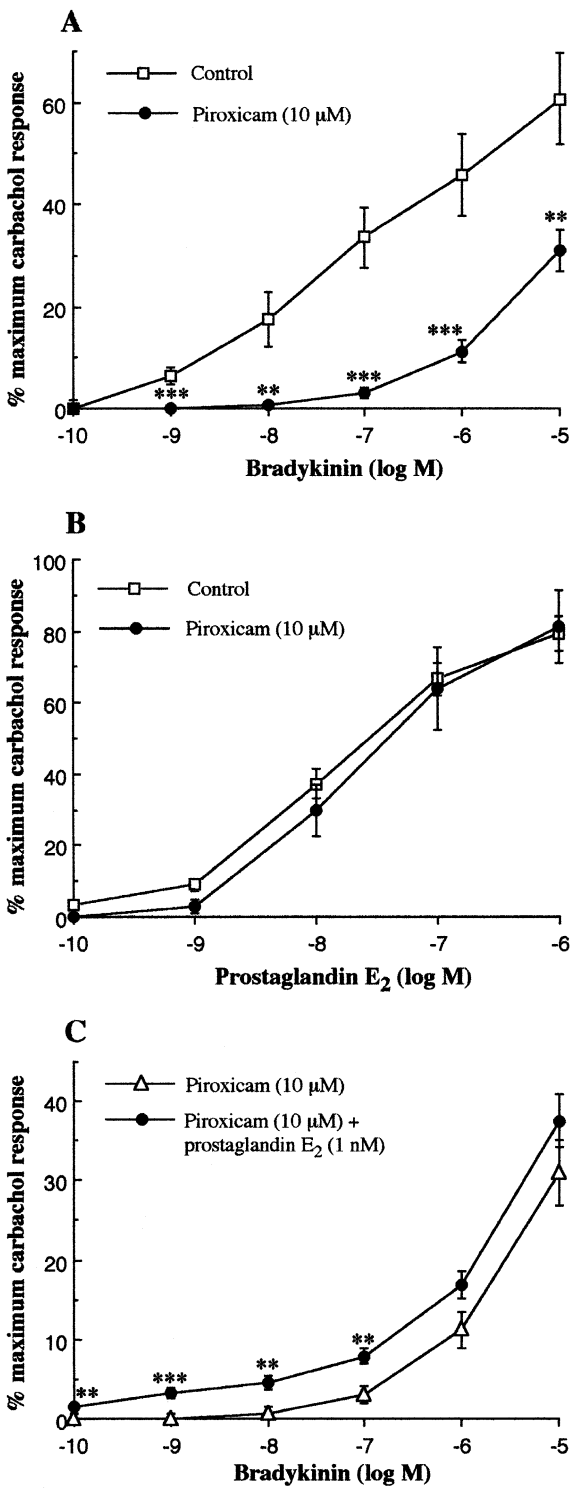

Fig. 1. Effect of piroxicam in guinea-pig gallbladder strips on the cumulative concentration–response curve to (A) bradykinin, (B) prostaglandin E_2 and (C) bradykinin in the presence of a threshold dose of prostaglandin E_2. Each point represents the mean ± S.E.M. of 8 to 14 experiments. Differs significantly from the control group: $^{*}P < 0.05$; $^{**}P < 0.005$; $^{***}P < 0.0005$.

3. Results

3.1. Effect of prostanoids on bradykinin induced contraction

Bradykinin (0.1 nM–10 μM) stimulated a concentration dependent sustained tonic contraction of guinea-pig gallbladder smooth muscle which was significantly attenuated by piroxicam (10 μM) (Fig. 1A). Exogenous prostaglandin E_2 (0.1 nM–1 μM) also stimulated gallbladder smooth muscle contraction. Pre-treatment with piroxicam (10 μM) had no effect on this response (Fig. 1B). In the presence of piroxicam (10 μM), a threshold concentration of prostaglandin E_2 (1 nM) significantly improved the capacity of gallbladder strips to respond to bradykinin ($P < 0.05$). This effect was more evident at lower concentrations of bradykinin (0.1 nM–0.1 μM) (Fig. 1C). Piroxicam caused a significant decrease in resting tone of gallbladder strips from 1.15 ± 0.11 to 0.61 ± 0.05 g ($n = 24$, $P < 0.0005$).

3.2. Effect of bradykinin on prostaglandin E_2 release

Bradykinin (10 μM) stimulated a significant increase in the release of prostaglandin E_2 from 1.4 ± 0.5 to 10.7 ± 4.1 pg/mg tissue ($n = 6$, $P < 0.05$) in guinea-pig gallbladder samples (Fig. 2). Piroxicam (10 μM) abolished both basal and bradykinin stimulated prostaglandin E_2 release ($P < 0.005$; data not shown).

3.3. Effect of a Ca^{2+}-free medium, nifedipine and SK&F 96365 on bradykinin induced contractions

Contractile responses to bradykinin were essentially abolished following incubation of guinea-pig gallbladder smooth muscle strips in a Ca^{2+}-free medium containing

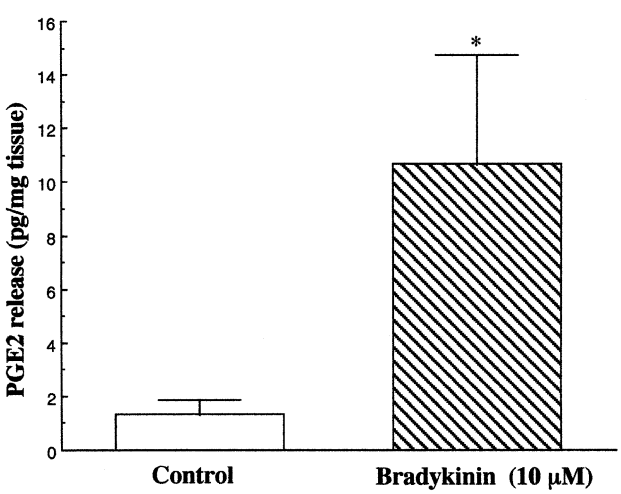

Fig. 2. Effect of bradykinin on prostaglandin E_2 release in guinea-pig gallbladder. Values represent the mean ± S.E.M. of six experiments. Differs significantly from the control group. $^{*}P < 0.05$.

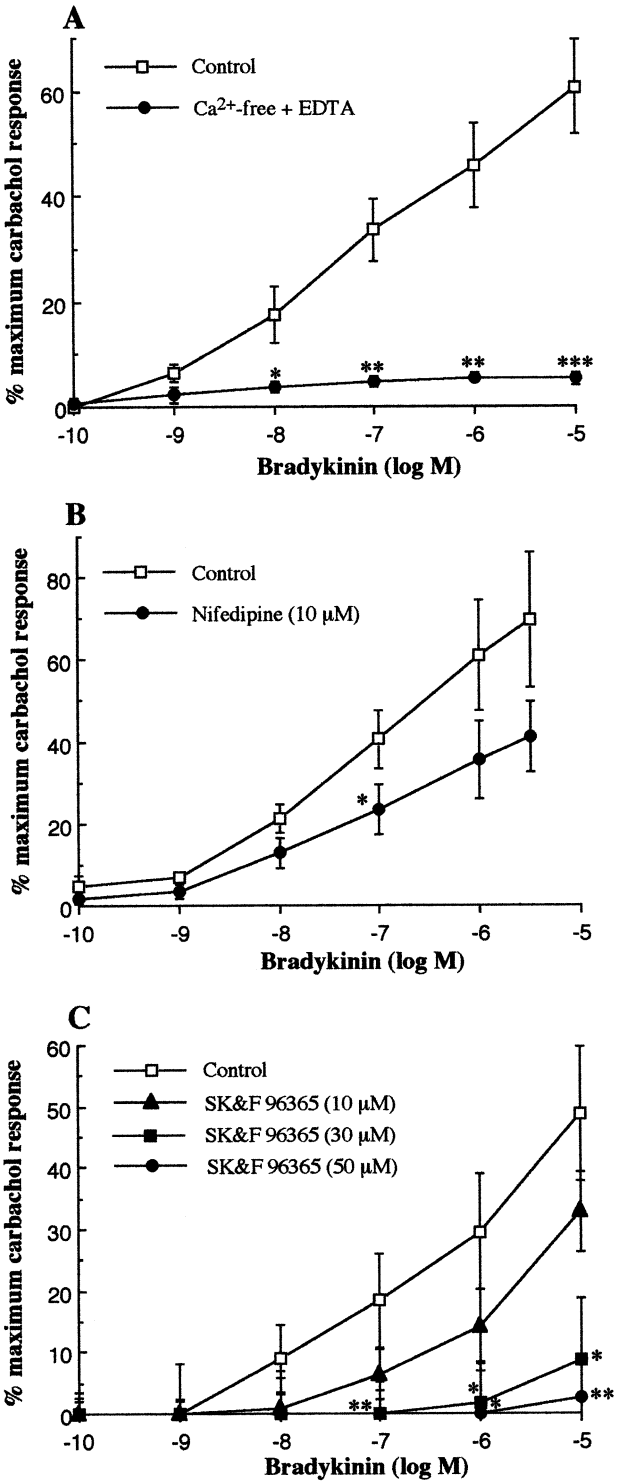

Fig. 3. Concentration–response curves for bradykinin in guinea-pig gallbladder strips generated in (A) a Ca^{2+}-free Krebs solution containing EDTA (1 mM), or in the absence or presence of (B) nifedipine or (C) SK&F 96365. Each point represents the mean ± S.E.M. of five to nine experiments. Differs significantly from the control group: $^{*}P < 0.05$; $^{**}P < 0.005$; $^{***}P < 0.0005$.

EDTA (1 mM) (Fig. 3A). Incubation in a Ca^{2+}-free medium resulted in a decrease in the resting tone from 1.15 ± 0.22 to 0.44 ± 0.06 g ($n = 7$, $P < 0.05$). Nifedipine (10 μM)

attenuated the contractile response to bradykinin ($P < 0.05$) (Fig. 3B). Nifedipine (30 and 50 μM) had no further inhibitory effects on the response to bradykinin (data not shown). Pre-incubation of guinea-pig gallbadder strips with nifedipine significantly decreased the resting tone from 0.52 ± 0.14 to 0.32 ± 0.12 g ($n = 14$, $P < 0.05$). SK&F 96365 (10–50 μM) decreased the contractile response to bradykinin in a dose-dependent fashion (Fig. 3C). The response was essentially abolished by the highest concentration of SK&F 96365 (50 μM), which had no effect on resting tone of the preparation. Fig. 4 shows typical tracings of the contractile response of guinea-pig isolated gallbladder strips to cumulative addition of bradykinin and the effects of nifedipine (10 μM) and SK&F 96365 (50 μM) on this response.

3.4. Effects of thapsigargin

Thapsigargin (1 μM) caused a sustained contraction in the presence of extracellular Ca^{2+} ($n = 4$). The contraction was $71 \pm 15\%$ of the maximum carbachol response and was therefore unsuitable for studying subsequent responses to bradykinin. Peak tension occurred approximately 6–8 min after the addition of thapsigargin to the bath and there was no significant decrease in response by 25 min (Fig. 5A). In strips bathed in a nominally Ca^{2+}-free medium (without EDTA), addition of thapsigargin (1 μM) produced a smaller increase in tension ($10 \pm 3\%$ of the maximum response to carbachol). Subsequent replacement of 2.5 mM Ca^{2+} in the presence of thapsigargin resulted in a rapid large sustained contraction ($96 \pm 7\%$ of the maximum response to carbachol) as shown in Fig. 5B.

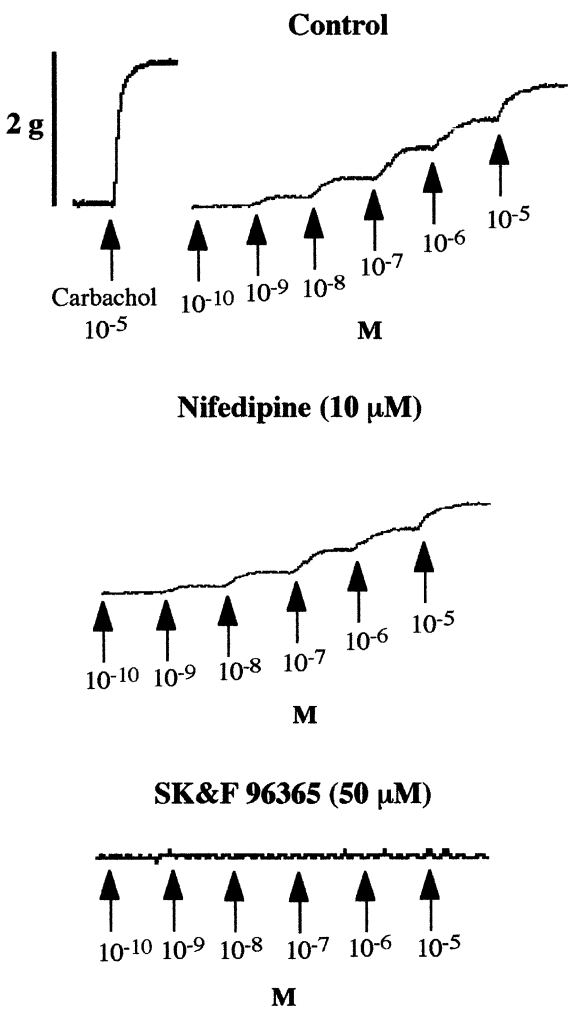

Fig. 4. Typical tracings showing the contractile response of guinea-pig isolated gallbladder strips to cumulative addition of bradykinin and the effects of nifedipine and SK&F 96365 on the response.

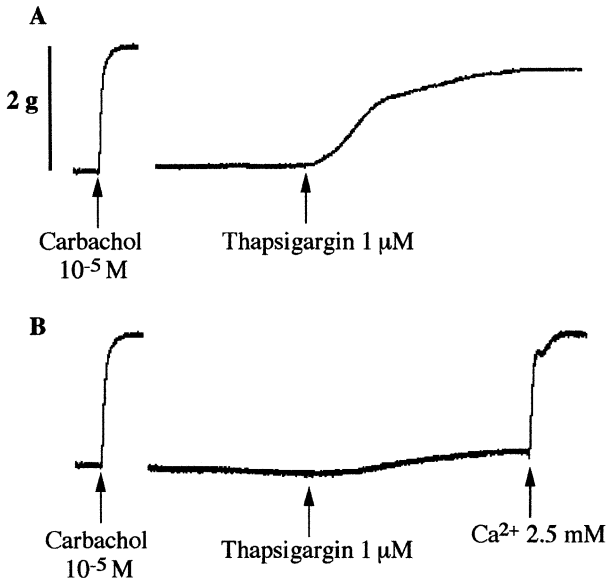

Fig. 5. Typical tracings showing the contractile response of guinea-pig gallbladder strips to thapsigargin in (A) normal Krebs solution and (B) Ca^{2+}-free Krebs. In (B) 2.5 mM Ca^{2+} was added 30 min after thapsigargin.

4. Discussion

The present study confirms that bradykinin elicits concentration dependent contractions of the guinea-pig gallbladder and that bradykinin induced contractions are dependent in part on prostanoid synthesis (Nakata et al., 1981; Woods and Baird, 1992; Cabrini et al., 1995; Falcone et al., 1993). Interestingly, in agreement with Falcone et al. (1993), we found no evidence for the high affinity phase of the contractile response to bradykinin reported by Cabrini et al. (1995). However, the separate studies may not be directly comparable due to methodological differences.

It has already been established that the gallbladder is capable of synthesizing prostaglandins of the E, F and I series (Booker and LaMorte, 1983; Longo et al., 1999; Grossman et al., 2000) and that bradykinin can stimulate

increased release of prostanoids from guinea-pig gallbladder (Bogar et al., 1999). We confirmed that bradykinin stimulates prostaglandin E_2 release and furthermore demonstrated that this release is abolished by piroxicam. We chose piroxicam, which is a cyclooxygenase inhibitor and has less effect upon intracellular Ca^{2+} transport than indomethacin (Burch et al., 1983).

Prostanoid synthesis is a feature of the action of several classes of spasmogen in gallbladder including endothelin (Nora et al., 2000) thrombin, trypsin and the peptide agonists of protease-activated receptors PAR-1 and PAR-2 (Tognetto et al., 2000). Thus, it was of interest to examine the nature of the interaction between prostaglandins and bradykinin. Although prostaglandin E_2 is a spasmogen in its own right, pre-treatment of gallbladder tissue with a threshold concentration of prostaglandin E_2 in the presence of piroxicam at a concentration we showed to abolish endogenous synthesis of prostaglandin E_2, partially restored the contractile responses to bradykinin. This information clearly indicates that prostaglandin E_2, at levels that show little spasmogenic activity, can enhance contractile responses to bradykinin. This phenomenon of Ca^{2+} sensitization of the contractile apparatus by agonist stimulation has been reviewed by Savineau and Marthan (1997). The priming effect of prostanoids on a range of biological responses to a range of stimuli has been previously documented. Studies investigating the sensitization of nociceptors by prostaglandins in skin and jejenum showed that the response to bradykinin, which was abolished in the presence of a cyclooxygenase inhibitor, was restored in the presence of sub-threshold levels of prostaglandin E_2 (Kindgen-Milles, 1995; Brunsden and Grundy, 1999). The mechanisms of such signaling interactions remain to be fully established but will be of interest in determining how kinins and prostanoids may contribute to gallbladder disease. Gallbladder inflammation and contractile dysfunction in a guinea pig model of bile duct ligation have been shown to be sensitive to indomethacin (Parkman et al., 2001) and drugs such as aspirin and other non-steroidal anti-inflammatory drugs have been proposed for prevention of gallstone formation and relief of associated pain (Babb, 1993). However, cyclooxygenase inhibitors may have complex and different effects upon gallbladder function in normal and diseased states (Greaves et al., 2000).

The present data indicate that bradykinin-mediated contractions in the guinea-pig gallbladder are highly dependent on extracellular Ca^{2+} influx, as its contractile responses were essentially abolished in a Ca^{2+}-free medium. Furthermore, the contractile responses elicited by bradykinin were partially attenuated by nifedipine but virtually abolished by SK&F 96365, suggesting that the contraction is largely due to extracellular Ca^{2+} influx via a receptor-mediated influx mechanism. In contrast, Cabrini et al. (1995) reported that bradykinin induced contraction of the guinea-pig gallbladder depends almost exclusively on Ca^{2+} release from intracellular sources. However, our results are in keeping with those of Cabrini and Calixto (1997) who showed that the contractile response to des-Arg^9-bradykinin, a selective bradykinin B_1 agonist, which like bradykinin was mediated via the bradykinin B_2 receptor, was completely dependent on an influx of extracellular Ca^{2+} partially through L-type Ca^{2+} channels.

There appears to be some tissue/site specificity regarding the Ca^{2+} requirements for bradykinin-induced contraction of smooth muscle. For example, dependence on extracellular Ca^{2+} influx via a receptor-mediated pathway has been demonstrated for bradykinin induced contraction in uterine and colonic smooth muscle (Wassdal et al., 1998; Zagorodnyuk et al., 1998). In contrast, bradykinin mediated contractions in the guinea-pig ileum, guinea-pig urinary bladder, rat vas deferens and rat uterus depend almost exclusively on extracellular Ca^{2+} influx via L-type voltage-sensitive dihydopyridine channels (Calixto, 1995).

Kinin B_1 and B_2 receptors belong to the superfamily of G-protein-coupled receptors which, upon activation, promote arachidonic release by phospholipase A_2 and phosphoinositol hydrolysis by phospholipase C leading to an increase in IP_3 (Farmer and Burch, 1992). IP_3 liberates Ca^{2+} from intracellular stores in the sarcoplasmic reticulum. Consequent depletion of the sarcoplasmic reticulum is the signal for the opening of store-operated channels in the plasma membrane through which Ca^{2+} can enter, thus providing so-called capacitative Ca^{2+} entry (Putney, 1990; Berridge, 1995). Until recently, capacitative Ca^{2+} entry was thought to be important only for refilling of the depleted sarcoplasmic reticulum and not as a source of activator Ca^{2+} for the contractile mechanism. However, current evidence indicates that this mechanism may be important for maintenance of sustained tone in some smooth muscle preparations (Gibson et al., 1998).

In order to assess the possible involvement of store-operated Ca^{2+} entry in the contractile response to bradykinin in guinea-pig gallbladder smooth muscle, we determined the effects of the sarcoplasmic reticulum Ca^{2+}-ATPase inhibitor, thapsigargin. Thapsigargin causes depletion of the sarcoplasmic reticulum stores in the absence of agonist activation of membrane receptors and can thus provide a distinction between Ca^{2+} entering through store-operated Ca^{2+} channels as opposed to receptor-operated Ca^{2+} channels.

Thapsigargin on its own produced strong and well maintained contractions of the the guinea-pig gallbladder that were dependent on extracellular Ca^{2+}. In Ca^{2+}-free solution, thapsigargin caused a slight contraction possibly mediated by Ca^{2+} release from internal stores. Subsequently, reintroduction of Ca^{2+} caused a large sustained contraction. These results demonstrate that mobilization of intracellular Ca^{2+} stores can initiate a contractile response in guinea-pig gallbladder smooth muscle. This is coupled to extracellular Ca^{2+} influx through store-operated Ca^{2+} channels and not receptor-operated channels since the contraction occurred without agonist stimulation.

In summary, we have shown that bradykinin induced contraction of guinea-pig gallbladder in vitro relies on prostanoid synthesis and that the contractile response to bradykinin, is modulated by prostaglandin E_2. We have also demonstrated that the bradykinin induced contractile response is highly dependent on extracellular Ca^{2+} influx. This influx occurs in part through voltage-operated Ca^{2+} channels which can be blocked by nifedipine, and mainly through store-operated Ca^{2+} channels, which can be blocked by SK&F 96365. This information may provide a mechanistic basis for the treatment of gallbladder disease by the inhibition of prostaglandin synthesis and the possible antagonism of selective Ca^{2+} channels.

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