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Multiple mechanisms of bradykinin-induced contraction in rat and guinea pig smooth muscles in vitro

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Abstract

Bradykinin caused graded contraction in the guinea pig ileum, trachea and urinary bladder and rat uterus and vas deferens in vitro. The order of potency (EC_{50}, nM) was: ileum (3) > uterus (5) > trachea (15) > vas deferens (41) > urinary bladder (52) and the maximal responses (percentage to 80 mM KCl) were: 152 ± 8 (ileum), 122 ± 6 (uterus), 97 ± 3 (urinary bladder), 75 ± 5 (trachea) and 33 ± 3 (vas deferens). Responses to bradykinin in guinea pig ileum and urinary bladder and rat vas deferens and uterus were markedly attenuated in Ca^{2+}-free medium with or without EGTA or by nicardipine, whereas those in guinea pig trachea depended almost exclusively on intracellular Ca^{2+} sources which were sensitive to ryanodine. Treatment of the animals with pertussis toxin only inhibited bradykinin-induced contraction of the rat uterus. Furthermore, the protein kinase C inhibitors, H_7 (5-isoquinolinysulfonyl-2-methyl-piperazine) and staurosporine, antagonized in a graded manner bradykinin responses in guinea pig ileum and trachea and rat vas deferens, indicating the possible dependence on activation of protein kinase C mechanisms, while responses of the rat uterus rely on coupling by a pertussis toxin-sensitive G protein. Thus, bradykinin acting at B_2 receptors may induce contractions in several smooth muscles from rat and guinea pig through activation of multiple second messenger pathways.

Keywords: Bradykinin; Uterus, rat; Vas deferens, rat; Trachea, guinea pig; Ileum, guinea pig; Urinary bladder, guinea pig; Ca^{2+}; G protein; Protein kinase C

1. Introduction

The nonapeptide bradykinin is generated in plasma from its percursor, the α_2-globulin named kininogen, by the actions of the enzyme kallikrein in response to tissue injury (Regoli and Barabé, 1980). Once released, bradykinin, acting as a local hormone, binds to specific membrane receptors and participates in a wide number of physiological and pathological states, such as vasodilatation, increase in vascular permeability, control of blood pressure and production of pain, and also participates in several inflammatory processes. Apart from these effects, bradykinin has been shown to induce potent contractile or relaxant effects in several vascular and nonvascular smooth muscles (see for review: Regoli and Barabé, 1980; Hall, 1992; Farmer and Burch, 1992).

Two receptor subtypes, denoted B_1 and B_2, have been defined on the basis of the rank order of potency of several bradykinin analogues, and more recently by the use of selective and highly potent kinin receptor antagonists. The bradykinin B_1 receptors seem to be restricted to some rabbit tissues and are normally expressed in pathological states or following tissue injury, and are characterized to exhibit greater affinity for the kinin metabolites des-Arg^9-bradykinin or des-Arg^{10}-kallidin (Lys-bradykinin) than for bradykinin. They are selectively antagonized by the B_1 receptor antagonists, des-Arg^9[Leu^8]bradykinin or des-Arg^{10}-[Leu^8]Lys-bradykinin. Conversely, bradykinin B_2 receptors are widely distributed throughout most tissues of mammalian species and mediate the majority of actions of kinin under normal conditions. The bradykinin B_2 re-

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ceptors present high affinity for bradykinin and kallidin and are selectively and competitively antagonized by several peptide B_2 receptor antagonists (reviewed by Hall, 1992; Farmer and Burch, 1992).

Although functional and biochemical studies suggest the possible heterogeneity of bradykinin B_2 receptors (reviewed by Hall, 1992; Farmer and Burch, 1992), recent evidence tends not to confirm this hypothesis, indicating that differences detected mainly on the basis of the potency for selective bradykinin B_2 receptor antagonists reflect species variations in bradykinin B_2 receptors (Hall et al., 1993; Hess et al., 1994). So far, the bradykinin B_2 receptor has been cloned from murine, rat and human species and they are members of the superfamily of guanine nucleotide binding protein (G protein) coupled receptors (McEachern et al., 1991; Eggrickx et al., 1992; Hess et al., 1992; 1994). However, the intracellular second messengers which mediate bradykinin responses are complex, and in most tissues, including smooth muscles, are less known. The effector systems activated by bradykinin depend on which enzymes or channels they are coupled to in a particular tissue. Thus, bradykinin-mediated responses involve both mobilization of extracellular Ca^{2+} influx, which enters the cell through membrane Ca^{2+} channels, and mobilization of intracellular Ca^{2+} stores (reviewed by Burch et al., 1993). The mechanism by which bradykinin mobilizes intracellular Ca^{2+} in most tissues involves the activation of phosphatidylinositol-specific phopholipase C, resulting in increases of cellular levels of inositol 1,4,5-trisphosphate and diacylglycerol. While inositol 1,4,5-trisphosphate releases intracellular Ca^{2+} from sarcoplasmatic reticulum, diacylglycerol directly activates protein kinase C (Farmer and Burch, 1992). In many tissues bradykinin's actions occur via activation of phospholipase A_2, resulting in the release of arachidonic acid which is metabolized to a variety of eicosanoid products (Burch et al., 1993). In other tissues, bradykinin-induced responses are associated with activation of Ca^{2+}-activated K^+ channels resulting in membrane hyperpolarization. Apart from these effects, bradykinin may also increase the cAMP or cGMP levels, although secondary to the release of prostanoids and nitric oxide (Hall, 1992).

The current study was undertaken to investigate, by the use of several pharmacological procedures, some of the second messenger transduction mechanisms involved in the contractile responses elicited by bradykinin in the guinea pig trachea, guinea pig urinary bladder, guinea pig ileum, rat vas deferens and rat uterus in vitro. These preparations of the two species were selected because bradykinin causes concentration-dependent contraction in all of them through activation of B_2 receptors and because they are widely used as pharmacological bioassays (Regoli and Barabé, 1980; Hall, 1992).

2. Material and methods

2.1. Tissue preparation

Guinea-pigs (200–350 g) and Wistar rats (200–350 g) of both sexes were lightly anesthetized with ether, killed by a blow on the head, and exsanguinated from the carotid arteries. The guinea pig trachea, guinea pig ileum, guinea pig urinary bladder, rat uterus and rat vas deferens were rapidly removed and carefully dissected free from adhering tissues. The female rats were treated 24 h before with estradiol benzoate (0.5 mg/kg s.c.). The rings of guinea pig trachea (3–4 mm wide) were opened and strips of guinea pig urinary bladder (10–15 mm in length) were set up in 5 ml jacketed organ baths containing Krebs-Henseleit solution maintained at 37°C, pH 7.2, and gassed continuously with 95% of O_2 and 5% of CO_2 with the following composition (mM): NaCl 113; KCl 4.7; $CaCl_2$ 2.5; $MgSO_4$ 1.1; KH_2PO_4 1.1 and glucose 11 and pH 7.4. The preparations were allowed to equilibrate for at least 60 min before drug addition under a resting tension of 1 g, during which time the bath solution was renewed every 15 min. Isometric contraction was recorded by means of an F 60 force transducer (Narco Biosystem). The epithelium of guinea pig trachea was removed by gently inserting the tip of a small pair of forceps into the luminal surface of the ring and rolling it back and forth on filter paper soaked with bathing medium. The absence of epithelium was confirmed by assessing the lack of relaxation response caused by bradykinin (100 nM) in preparations under spontaneous tonus (Schlemper and Calixto, 1994). The guinea pig ileum (20 mm in length), and the whole rat vas deferens and rat uterus (15–20 mm in length) were set up in 5 ml organ baths containing Tyrode (37°C) solution, modified Krebs solution (30°C) or De Jalon solution (30°C), respectively. Preparations were bubbled with air under 1 g (guinea pig ileum and rat uterus) and 0.5 g of load (rat vas deferens). The isotonic contractions were recorded by means of a light lever (6-fold amplification) writing on a kymograph. The physiological solutions had the following composition (mM): De Jalon solution (NaCl 154; KCl 4.7; $CaCl_2$ 0.3; $MgCl_2$ 1.4; $NaHCO_3$ 1.7 and glucose 5.5), Tyrode solution (NaCl 137; KCl 2.7; $CaCl_2$ 1.8; $MgCl_2$ 1.0; $NaHCO_3$ 11.9; NaH_2PO_4 0.4 and glucose 5.5), and modified Krebs solution (NaCl 136.8; KCl 5.6; $CaCl_2$ 1.3; NaH_2PO_4 0.4; $NaHCO_3$ 14.8 and glucose 5.5). The pH was ajusted to 7.2. Preparations were allowed to equilibrate for at least 60 min before the experiments were started, and during this period the bath solution was changed every 15 min. Responses to bradykinin in all tissues were determined in the presence of captopril (3 μM) to avoid degradation by the action of kininase II. Confirming our previous findings (Medeiros and Calixto,

1993), no significant differences were observed in the responsiveness to bradykinin when the experiments were carried out in preparations aired with carbogen or bubbled with air ($n = 3$, results not shown).

2.2. Concentration-response curves

Following the appropriate equilibration period, preparations were challenged with 80 mM KCl (prepared by equimolar replacement of NaCl by KCl in the medium) to evaluate the maximal response of each preparation. After washout, replacement with normal medium, and return to baseline, complete cumulative concentration-response curves were obtained for bradykinin (0.1 nM to 10 μM) in the absence or in the presence of several drugs. In order to avoid or minimize bradykinin desensitization, only one complete bradykinin concentration-response curve was obtained in the guinea pig trachea and in the rat vas deferens. In the other preparations, 2–3 complete cumulative concentration-response curves were obtained for bradykinin at 30-to 60-min intervals between curves. The cumulative concentration-response curves for bradykinin were obtained by a stepwise increase of the agonist in approximately 0.5 log unit increments. Each drug concentration of the cumulative curve was added when the effect of the preceding one had reached its maximum.

2.3. Influence of calcium

To assess the contribution of extracellular Ca^{2+} to bradykinin-mediated contractions before obtaining a complete concentration-response curve for bradykinin, preparations were set up in normal physiological salt solution, and after obtaining the contraction response for KCl (80 mM), they were placed for 30 min in physiological solution free of Ca^2 or in Ca^{2+}-free solution containing EGTA (1 mM). To investigate the roles played by L-type Ca^{2+} channels in bradykinin-mediated contraction, in some experiments the preparations were incubated with the L-type Ca^{2+} channel blocker nicardipine (1 nM to 1 μM) for 20 min and a new complete cumulative concentration-response curve for bradykinin was obtained in its presence. As bradykinin-induced contraction in guinea pig trachea was only partially affected in Ca^{2+}-free medium, we next examined the possible contribution of Ca^{2+} from intracellular sources to bradykinin-mediated contractions. To this end, complete concentration-response curves for bradykinin were obtained in the absence or in the presence of the alkaloid ryanodine (10 and 30 μM) incubated with guinea pig trachea 20 min beforehand, either in normal medium or in Ca^{2+}-free solution.

2.4. Effect of protein kinase C

The possible role of protein kinase C in the contractile response elicited by bradykinin was evaluated by the use of the protein kinase C inhibitors, H_7 and staurosporine, or the selective protein kinase C activator, phorbol ester. Following the equilibration period, complete concentration-response curves were obtained for bradykinin in the absence or in the presence of H_7 (10 and 30 μM), staurosporine (1–100 nM) or phorbol ester (1 μM), incubated with the tissues for 30 min in the case of H_7 and staurosporine and for 60 min for phorbol ester.

2.5. Influence of pretreatment with pertussis toxin

The possible participation of G_i-or G_o-coupled protein in bradykinin-mediated contractions in these preparations was also investigated. Three days before the experiments, animals under ether anesthesia received an intravenous injection of pertussis toxin (10 μg/kg) (Eglen et al., 1987). The responsiveness to bradykinin of preparations taken from pertussis toxin-treated animals was compared to that of control segments from saline-treated animals. Confirming our previous observations (Calixto and Medeiros, 1991), the pertussis toxin treatment (10 μg/kg i.v.) inhibited carbachol-induced negative inotropic and chronotropic activities in isolated guinea pig and rat hearts ($n = 4$, results not shown).

2.6. Drugs

The following drugs were used: bradykinin, H_7 (5-isoquinolinylsulfonyl-2-methyl-piperazine), nicardipine, phorbol 12-myristate 13-acetate, captopril, pertussis toxin (Sigma Chemical Co., St. Louis, MO, USA), ryanodine (Research Biochemicals, Natick, MA, USA). The stock solution of these drugs (1–10 mM) was prepared as follows: bradykinin, H_7 and captopril in saline 0.9%; all other drugs were diluted in absolute ethanol. Pertussis toxin was dissolved in physiological buffer solution, pH = 7.4. The final ethanol bath concentration was less than 0.2% and had no effect on the tissues' responsiveness to bradykinin. Appropriate parallel control experiments were carried out with only the vehicle used to dilute these drugs. All experiments carried out with nicardipine were protected from light to avoid its degradation.

2.7. Statistical analysis

The contractile responses for bradykinin are presented as percentages of the response to 80 mM KCl. The EC_{50} values (i.e. the concentration of bradykinin causing half-maximal contractile response) were deter-

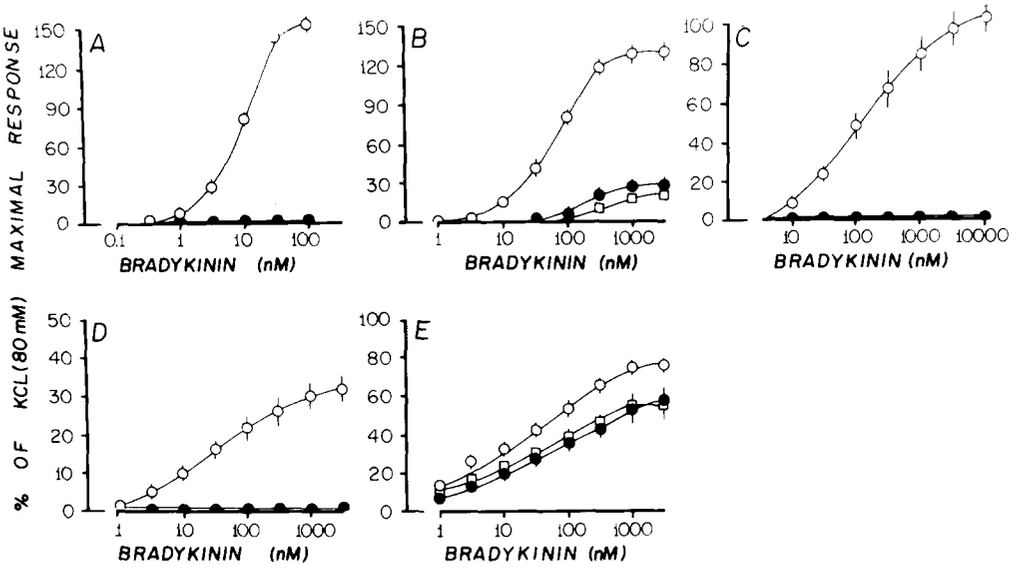

Fig. 1. Mean concentration-response curve for bradykinin in several nonvascular smooth muscles from (A) guinea pig ileum, (B) rat uterus, (C) guinea pig urinary bladder, (D) rat vas deferens and (E) guinea pig trachea. Control responses (○) or responses obtained in tissues maintained in Ca^{2+}-free solution (●) or in Ca^{2+}-free solution plus EGTA (1 mM) (□). Each point represents the mean of 5–7 experiments and the vertical lines indicate the S.E.M.

mined for individual experiments from complete bradykinin concentration-response curves by the use of the least-squares method. Only one antagonist was tested in each preparation. The EC_{50} are presented as the geometric means accompanied by their respective 95% confidence limits. All other values shown represent the mean ± S.E.M. Statistical significance was assessed by unpaired Student's *t*-test. Differences between groups were considered to be significant at $P < 0.05$.

3. Results

3.1. Concentration-response curves for bradykinin

Cumulative addition of bradykinin (0.1 nM to 10 μM) caused a concentration-dependent contractile response in all studied preparations. With the exception of the actions of bradykinin in guinea pig trachea and guinea pig urinary bladder, which were characterized by slowly developed sustained tonic contraction,

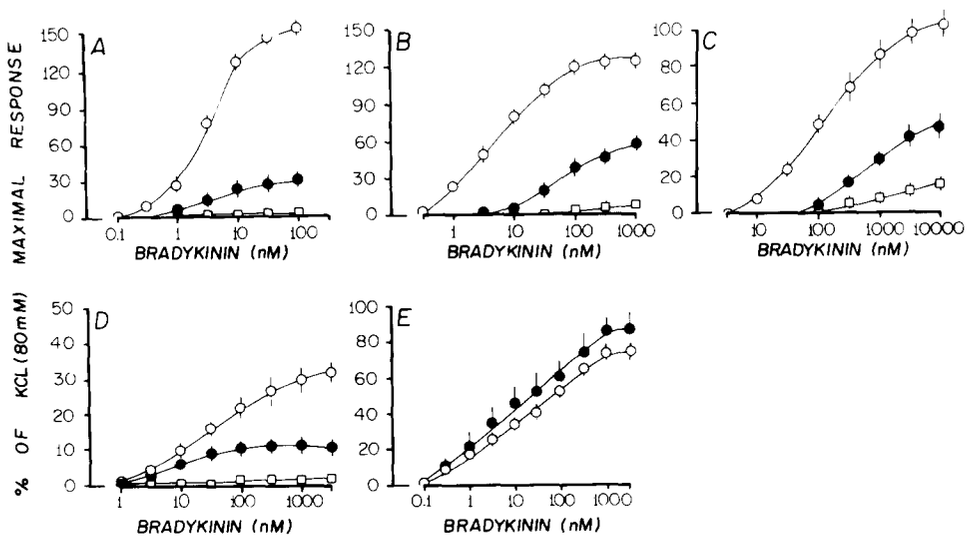

Fig. 2. Mean concentration-response curve for bradykinin in several nonvascular smooth muscles from (A) guinea pig ileum, (B) rat uterus, (C) guinea pig urinary bladder, (D) rat vas deferens and (E) guinea pig trachea. Control responses (○) or responses obtained in the presence of nicardipine: (A) 0.01 μM (●), 0.1 μM (□); (B) 0.1 μM (●), 1 μM (□); (C) 0.1 μM (●), 1 μM (□); (D) 0.001 μM (●), 0.01 μM (□); and (E) 1 μM (●). Each point represents the mean of 6–8 experiments and the vertical lines indicate the S.E.M.

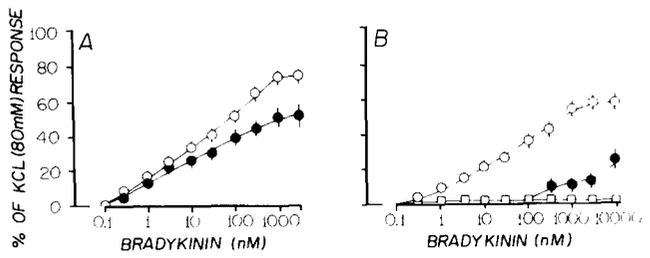

Fig. 3. Mean concentration-response curve for bradykinin in the guinea pig isolated trachea obtained in normal medium (A) in the absence (○) or in the presence of ryanodine (30 μM, ●), or responses obtained in Ca^{2+}-free solution (B) in the absence (○) or in the presence of 10 μM (●) or 30 μM (□) of ryanodine. Each point represents the mean of 5 experiments and the vertical lines indicate the S.E.M.

bradykinin-induced contractions in guinea pig ileum, rat uterus and rat vas deferens were characterized by a rapid phasic response. The relative rank order of potency (EC_{50}, nM and 95% confidence limits) was: guinea pig ileum, 3.4 (2.5–10.8) > rat uterus 5.3 (3.4–8.5) > guinea pig trachea 15.5 (11.4–20.7) > rat vas deferens 41.7 (31.3–62.8) > guinea pig urinary bladder 52.3 (38.6–69.2). The maximal contractions (in percentage of 80 mM KCl contractions, mean ± S.E.M.) were: 152 ± 8 (guinea pig ileum), 122 ± 6 (rat uterus); 97 ± 3 (guinea pig urinary bladder); 75 ± 5 (guinea pig trachea) and 33 ± 3 (rat vas deferens).

3.2. Effect of calcium removal, nicardipine and ryanodine on bradykinin-induced contraction

The contractile responses elicited by bradykinin in the guinea pig ileum, guinea pig urinary bladder and rat vas deferens were completely abolished when the preparations were maintained in Ca^{2+}-free medium for 30 min (Fig. 1A, C and D). Although bradykinin-induced contraction in rat uterus was also markedly depressed in Ca^{2+}-free medium (percentage of inhibition, mean ± S.E.M. of 76 ± 5), its response was not significantly depressed any further in Ca^{2+}-free medium plus EGTA (1 mM) (81 ± 4%) (Fig. 1B). Conversely, bradykinin-induced contractions in guinea pig trachea were partially but significantly inhibited ($P < 0.05$) (28 ± 4%) when preparations were placed in Ca^{2+}-free medium. Similar inhibition for bradykinin-induced contraction (26 ± 4%) was observed when guinea pig tracheas were placed in Ca^{2+}-free medium containing EGTA (1 mM) (Fig. 1E). Preincubation of the preparations with nicardipine (0.001–1 μM) had no effect on the tone of the preparations (results not shown), but caused a potent and graded inhibition of bradykinin-induced contraction in guinea pig ileum, rat uterus, guinea pig urinary bladder and rat vas deferens (Fig. 2A, B, C and D). The inhibition was 100; 92 ± 4; 85 ± 3 and 93 ± 3%, respectively. Nicardipine was most potent in inhibiting bradykinin-induced contraction in rat vas deferens followed by guinea pig ileum > rat uterus = guinea pig urinary bladder. However, nicardipine up to 1 μM failed to affect bradykinin-induced contraction in guinea pig trachea (Fig. 2E), but consistently antagonized KCl-induced contraction (control response of 3.54 ± 0.32 g and response in the presence of nicardipine of 1.46 ± 0.28 g, $P < 0.05$, $n = 5$). Preincubation of guinea pig trachea, maintained in normal Krebs solution with ryanodine (30 μM), caused partial but significant inhibition of bradykinin-induced con-

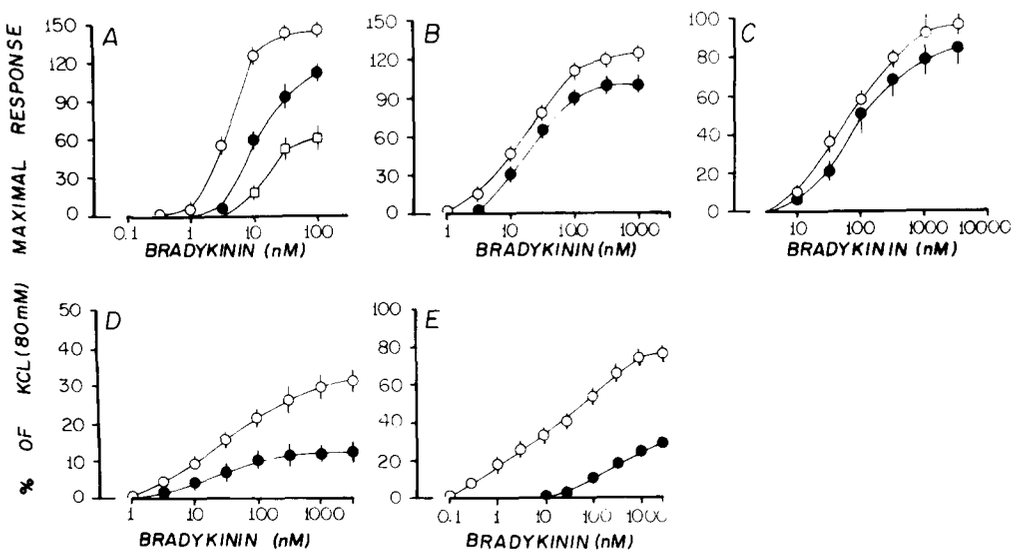

Fig. 4. Mean concentration-response curve for bradykinin in several nonvascular smooth muscles from (A) guinea pig ileum, (B) rat uterus, (C) guinea pig urinary bladder, (D) rat vas deferens and (E) guinea pig trachea. Control responses (○) or responses obtained in the presence of H_7 (A) 10 μM (●) and 30 μM (□); or 30 μM (●) (B, C, D and E). Each point represents the mean of 6–7 experiments and the vertical lines indicate the S.E.M.

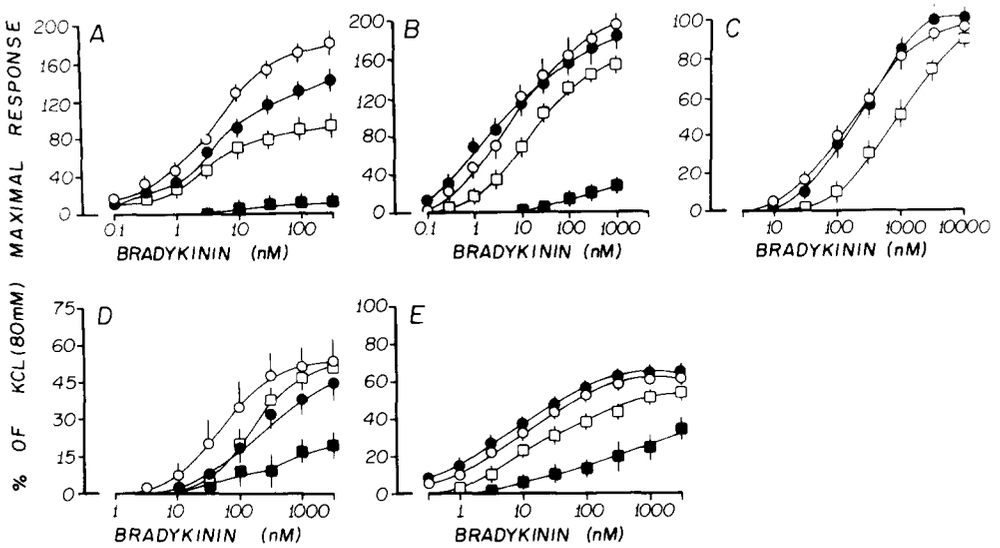

Fig. 5. Mean concentration-response curve for bradykinin in several nonvascular smooth muscles from (A) guinea pig ileum, (B) rat uterus, (C) guinea pig urinary bladder, (D) rat vas deferens and (E) guinea pig trachea. Control responses (○) or responses obtained in the presence of staurosporine. (A) 3 nM (●), 10 nM (□) and 30 nM (■); (B) 3 nM (●), 30 nM (□) and 100 nM (■); (C) 10 nM (●) and 100 nM (□); (D) 1 nM (●), 10 nM (□) and 100 nM (■); and (E) 1 nM (●), 10 nM (□) and 100 nM (■). Each point represents the mean of 5–7 experiments and the vertical lines indicate the S.E.M.

traction (33 ± 3%) ($P < 0.05$) (Fig. 3A). However, when this alkaloid (10 μM and 30 μM) was added to the guinea pig trachea maintained in Ca^{2+}-free medium, it markedly prevented bradykinin-mediated contraction in a concentration-dependent manner (Fig. 3B). At 30 μM, ryanodine completely abolished bradykinin-induced contraction in this preparation (Fig. 3B), leaving the contraction induced by KCl unaffected (control response of 3.41 ± 0.30 g versus 2.95 ± 0.32 g in the presence of ryanodine, $P > 0.05$, $n = 5$).

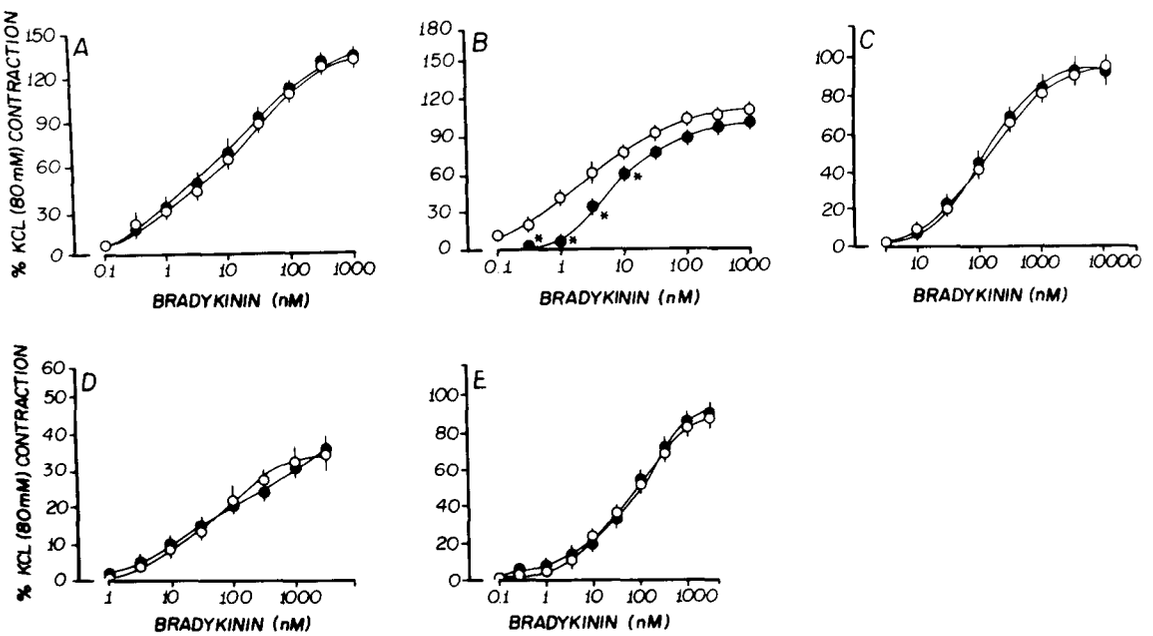

Fig. 6. Mean concentration-response curves for bradykinin in several nonvascular smooth muscles from (A) guinea pig ileum, (B) rat uterus, (C) guinea pig urinary bladder, (D) rat vas deferens and (E) guinea pig trachea. Response obtained in saline-treated animals (○) or responses obtained in animals pretreated with pertussis toxin (10 μg/kg i.v., 3 days prior to experiments) (●). Each point represents the mean of 5–7 experiments and the vertical lines indicate the S.E.M.

3.3. Effect of protein kinase C inhibitors and activator on bradykinin action

Preincubation of guinea pig ileum, rat vas deferens and guinea pig trachea with H_7 (10 and 30 μM), a protein kinase C inhibitor, failed to affect the tone of the tissue (results not shown), but inhibited bradykinin-mediated contractions, with inhibition at 30 μM of $59 \pm 4\%$, $61 \pm 3\%$ and $62 \pm 4\%$, respectively. Interestingly, H_7 up to 30 μM failed to significantly affect bradykinin-elicited contraction in rat uterus and in guinea pig urinary bladder (Fig. 4B and C) as well as KCl-mediated contraction in all studied preparations (results not shown, $n = 4–5$ experiments).

Staurosporine (1–100 nM), another protein kinase C inhibitor, also did not affect the tonus of the preparations (not shown), but caused graded and significant inhibition of bradykinin-induced contraction in guinea pig ileum, rat vas deferens and guinea pig trachea (Fig. 5A, D and E). The inhibition of bradykinin (0.1 nM to 10 μM) responses caused by 30–100 nM of staurosporine was 94 ± 4; 65 ± 7 and 58 ± 8, respectively. In contrast, only higher concentration of staurosporine (100 nM) inhibited bradykinin-induced contractions in rat uterus ($86 \pm 6\%$ of inhibition) and caused a marked displacement to the right of the bradykinin-induced contractile response in guinea pig urinary bladder (Fig. 5B and D). As reported for H_7, the highest effective concentration used of staurosporine (100 nM) did not significantly affect the contraction induced by KCl in all studied tissues (results not shown, $n = 4–5$ experiments).

Interestingly, short-term exposure (60 min) of all preparations to phorbol ester (1 μM), a diterpene known to activate protein kinase C, did not significantly modify bradykinin-mediated contractions in any studied tissues (results not shown, $n = 6–7$ experiments). In addition, phorbol ester up to 1 μM failed to affect the tonus of the studied preparations (results not shown).

3.4. Influence of pertussis toxin pretreatment

The pretreatment of animals with pertussis toxin (10 μg/kg i.v.) 3 days prior to the experiments did not result in any significant change of bradykinin-mediated contractions in guinea pig ileum, guinea pig trachea, guinea pig urinary bladder or rat vas deferens (Fig. 6A, C, D and E). However, the same treatment caused a small but significant (about 3-fold) displacement to the right of bradykinin-mediated contractions of rat uterus. The EC_{50} of pertussis toxin-treated rats was 9.2 (7.2–12.7) nM and that of saline-treated rats was 3.2 (1.1–5.8) nM (Fig. 6B). However, the maximal response elicited by bradykinin was not significantly affected in pertussis toxin-treated preparations (Fig. 6B).

4. Discussion

The results of the present study demonstrate that bradykinin caused potent and concentration-dependent contractile responses in guinea pig ileum, guinea pig urinary bladder, guinea pig trachea, rat uterus and rat vas deferens, but the second messenger mechanisms underlying its responses markedly differed among the preparations. Bradykinin exhibited similar potency in eliciting contraction in guinea pig ileum, rat uterus and guinea pig trachea, but it was about 8–10 times less potent in inducing contraction in rat vas deferens and guinea pig urinary bladder. In addition, the maximal responses to bradykinin also markedly differed among the studied preparations, being greater in guinea pig ileum, followed by rat uterus > guinea pig urinary bladder > guinea pig trachea ≫ rat vas deferens.

Of interest are the results indicating that bradykinin-mediated contractions in guinea pig ileum, guinea pig urinary bladder, rat vas deferens, and rat uterus rely almost exclusively on extracellular Ca^{2+} influx, as its contractile responses were abolished in Ca^{2+}-free medium. Moreover, the contractile responses elicited by bradykinin in rat vas deferens, guinea pig ileum and guinea pig urinary bladder were very sensitive to the action of nicardipine, suggesting that bradykinin induces Ca^{2+} mobilization from extracellular medium in such preparations via L-type voltage-sensitive dihydropyridine channels. Such results differ somewhat from similar experiments carried out with bradykinin in aorta and jugular vein from rabbits (Calixto and Medeiros, 1992), in circular muscle from guinea pig ileum (Calixto and Medeiros, 1991) and guinea pig gallbladder (Cabrini et al., 1995), which were quite resistant to the action of nicardipine. These results indicate that bradykinin-induced contractions in the latter preparations presumably involve mechanisms other than the activation of voltage-sensitive L-type Ca^{2+} channels. Bradykinin-mediated contraction in guinea pig trachea relies only partially on extracellular Ca^{2+} influx, as indicated by the fact that its contractile response was resistant to Ca^{2+}-free medium or Ca^{2+}-free solution plus EGTA. Furthermore, nicardipine, at a concentration that markedly antagonized KCl-induced contraction in guinea pig trachea, failed to interfere with bradykinin-induced contraction in this preparation. Taken together, such results are consistent with the view that bradykinin-mediated contraction in guinea pig trachea is mainly associated with the release of Ca^{2+} from intracellular store sources. This hypothesis was further investigated by examining the effect of the neutral alkaloid ryanodine, which has been recently demonstrated to bind specifically with ryanodine receptors on the sarcoplasmatic reticulum. These receptors are widely distributed in the peripheral and in the central nervous system of several mammalian species

(reviewed by Sorrentino and Volpe, 1993; McPherson and Campbell, 1993; Meissner, 1994). This alkaloid has been reported to affect muscle contraction either by closing or opening the sarcoplasmatic reticulum Ca^{2+} channels. Thus, ryanodine, when added to the guinea pig trachea maintained in Ca^{2+}-free solution, and to a lesser degree in normal medium, caused significant inhibition of bradykinin-mediated contraction in guinea pig trachea, thus further supporting our previous notion that bradykinin-induced contraction in guinea pig trachea appears to be coupled to activation of phospholipase C and generation of inositol 1,4,5-trisphosphate. The action of ryanodine on bradykinin-mediated contraction in guinea pig trachea seems to be quite selective, as at the higher concentration used this alkaloid did not significantly affect KCl-induced contractions. Similar inhibition by ryanodine of bradykinin-induced contraction via stimulation of B_2 receptor has been demonstrated in the guinea pig gallbladder in vitro (Cabrini et al., 1995). Recently, Yang et al. (1994) reported very similar results indicating that bradykinin acting on B_2 receptors increases the intracellular Ca^{2+} concentration in canine trachea smooth muscle cells through activation of B_2 receptors. This bradykinin effect involves release of Ca^{2+} from intracellular Ca^{2+} stores followed by the influx of external Ca^{2+}. One ryanodine receptor, termed $\beta 4$, has been demonstrated to be coupled to bradykinin receptor activation in mink lung epithelial cells (Giannini et al., 1992).

Another interesting aspect investigated in the present study was the fact that the contractile responses elicited by bradykinin in guinea pig ileum, rat vas deferens and guinea pig trachea and partially in rat uterus, but not in guinea pig urinary bladder, were markedly reduced by the protein kinase C antagonists H_7 and staurosporine, staurosporine being markedly (50- to 100-fold) more potent. Although some nonspecific effects of both protein kinase C inhibitors have been reported (Hidaka and Kobayashi, 1992; Ruegg and Burguess, 1989), in our models their actions seem to be quite selective, as at the concentration that they were effective in antagonizing bradykinin-mediated contractions, neither drug significantly interfered with KCl-mediated responses. Similar inhibition of bradykinin-mediated contraction by protein kinase C inhibitors has been demonstrated previously in circular muscle from guinea pig ileum (Calixto and Medeiros, 1991), rabbit aorta and jugular vein (Calixto and Medeiros, 1992) and rat portal vein (Campos and Calixto, 1994), but not in guinea pig gallbladder (Cabrini et al., 1995). These findings suggest that protein kinase C-dependent mechanisms play an important role in the action of bradykinin in such preparations, possibly by stimulation of phosphatidylinositide-specific phospholipase C turnover and formation of diacylglycerol (reviewed by Hall, 1992; Farmer and Burch, 1992). However, there are marked variations in the sensitivity of protein kinase C inhibitors among tissues and animal species. We did not further explore the reason for the differences in the present study. However, recently reported results revealed a greater differential sensitivity of protein kinase C inhibitors when analysed against different isoforms of this protein (Shimamoto et al., 1993; Wilkinson et al., 1993). Thus, the possible existence of isoforms of protein kinase C among preparations might explain, at least in part, such discrepant data. Surprisingly, the short-term (60 min) activation of protein kinase C with the selective protein kinase C activator, phorbol 12-myristate 13-acetate, failed to affect bradykinin-mediated contractions and did not cause any effect per se on the tonus of the preparations. It is possible that the lack of effect of phorbol on the basal tone as well as in bradykinin-mediated contraction in such tissues, in contrast to that reported in other smooth muscle preparations (Rasmussen et al., 1987; Guimarães et al., 1992), is due to the short period of incubation. Alternatively, the bradykinin-induced contraction mediated by activation of B_2 receptors in these preparations could involve a complex mechanism of action, and activation of protein kinase C alone might not be sufficient to elicit contraction. Additional experiments are clearly required to clarify this point.

It is well established that tissue sensitivity to bacterial toxins, such as pertussis toxin, a toxin that is known to catalyse the adenosine 5-diphosphate ribosylation of G_o- and G_i-proteins, has long been used as a criterion to investigate the possible involvement of a class of G proteins that couple neurotransmitter responses in many cell types (Murayama and Ui, 1983; Ui, 1986). Thus, we also assessed whether guanine nucleotide regulatory proteins G_o and/or G_i couple bradykinin-mediated contractions in these preparations. The treatment of animals with pertussis toxin (10 μg/kg i.v.) 3 days before the experiments failed to affect bradykinin-mediated contractions in guinea pig ileum, guinea pig urinary bladder, guinea pig trachea and rat vas deferens. These findings are consistent with the view that coupling of bradykinin receptors to a phospholipase C and to Ca^{2+} entry in such tissues is probably mediated by G proteins which are presumably distinct from the known pertussis toxin-sensitive G_i or G_o. However, bradykinin-induced contraction in the rat uterus was partially but significantly inhibited in animals pretreated with pertussis toxin, suggesting that G_i and/or G_o protein modulates bradykinin-mediated contraction in this preparation. These findings are in accordance with recently reported data indicating that bradykinin B_2 receptors are coupled to pertussis toxin-sensitive G proteins (G_i2, $G_i2\alpha$, $G_i3\alpha$ and G_o) in rat myometrium (Tanfin and Harbon, 1987; Milligan et al., 1989; Liebmann et al., 1990; Tanfin et al., 1991). Of particular

interest are the previous findings showing that the G_i protein levels in the rat myometrium change during gestation, and that previous treatment with pertussis toxin on day 12 of pregnancy attenuated cyclic AMP responses, suggesting an inhibitory involvement of G_i proteins in this process (Tanfin and Harbon, 1987). Several studies have directly or indirectly suggested that bradykinin-mediated responses in many tissues are susceptible to modulation by pertussis toxin-sensitive (G_i or G_o) proteins (Liebmann et al., 1990; Bozou et al., 1989; Ewald et al., 1989; Gil-Longo et al., 1993), while in other tissues bradykinin-mediated responses seem to be unaffected by pertussis toxin (Burch and Axelrod, 1987; Kremer et al., 1987; Perney and Miller, 1989; Calixto and Medeiros, 1991; Liao and Homcy, 1994; Wilkb-Laszczak et al., 1994).

In summary, the results of the current study provide consistent evidence indicating that the post-receptor mechanisms involved in bradykinin-mediated contractions of nonvascular smooth muscle preparations from guinea pigs and rats are regulated by multiple mechanisms. Although all bradykinin responses in these tissues are thought to be mediated by activation of B_2 receptors (Hall, 1992; Farmer and Burch, 1992), the contractile responses induced by bradykinin in rat vas deferens, guinea pig ileum, guinea pig urinary bladder and rat uterus are largely dependent on the influx of Ca^{2+} from the extracellular medium, which is very sensitive to inhibition by nicardipine, a voltage-sensitive L-type Ca^{2+} channel antagonist. In contrast, bradykinin-mediated contraction in guinea pig trachea relies much less on influx of extracellular Ca^{2+} and depends strongly on the release of Ca^{2+} from intracellular stores, a mechanism which was abolished by ryanodine. Furthermore, bradykinin-mediated contractions in guinea pig ileum, rat vas deferens and guinea pig trachea seem to involve, at least in part, activation of protein kinase C-dependent mechanisms. Finally, we have also provided functional evidence indicating that bradykinin-induced contraction in rat uterus, but not in guinea pig ileum, guinea pig trachea, guinea pig urinary bladder or rat vas deferens, is mediated by a mechanism regulated by pertussis toxin-sensitive G_i and/or G_o coupled proteins. Such differences in the mechanisms of bradykinin-mediated contractions in these preparations may indicate that this peptide, acting through stimulation of bradykinin B_2 receptors on smooth muscles, can activate multiple signalling pathways, which in turn control muscle contraction. Furthermore, it cannot be ruled out that such differences in bradykinin responses can be partially attributed to the small changes in bradykinin sequence of B_2 receptors reported to occur among animal species (Hall et al., 1993; Hess et al., 1994).

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References

Bozou, J.C., F. De Nadai, J.P. Vicent and P. Kitabgi, 1989, Neurotensin, bradykinin and somatostatin inhibit cAMP production in neuroblastome N1E-115 cells via both pertussis toxin sensitive and insensitive mechanisms, Biochem Biophys. Res. Commun. 161, 1144.

Burch, R.M. and J. Axelrod, 1987, Dissociation of bradykinin-induced formation from phosphatidylinositol turnover in Swiss 3T3 fibroblast: evidence for G protein regulation of phospholipase A_2, Proc. Natl. Acad. Sci. USA 84, 6374.

Burch, R.M., D.J. Kyle and T.M. Stormenn, 1993, Transduction of bradykinin signals, in: Molecular Biology and Pharmacology of Bradykinin Receptors, eds. R.M. Burch and R.G. Austin (Landes Co.) p. 33.

Cabrini, D.A., A.M. Silva and J.B. Calixto, 1995, Mechanisms of bradykinin-induced contraction of the guinea-pig gallbladder in vitro, Br. J. Pharmacol. 114, 1549.

Calixto, J.B. and Y.S. Medeiros, 1991, Characterization of bradykinin mediating pertussis toxin-insensitive biphasic response in circular muscle of the isolated guinea pig ileum, J. Pharmacol. Exp. Ther. 259, 659.

Calixto, J.B. and Y.S. Medeiros, 1992, Effect of protein kinase C and calcium on bradykinin-mediated contractions of rabbit vessels, Hypertension 19, II-87.

Campos, A.H. and J.B. Calixto, 1994, Mechanism involved in the contractile responses of kinins in rat portal vein rings: mediation by B_1 and B_2 receptors, J. Pharmacol. Exp. Ther. 268, 902.

Eggrickx, D., E. Raspe, D. Bertrand, G. Vassart and M. Parmentier, 1992, Molecular cloning expression and pharmacological characterization of a human bradykinin B_2 receptor gene, Biochem. Biophys. Res. Commun. 187, 1306.

Eglen, R.M., M.M. Huft, W.W. Montgomery and R.L. Whiting, 1987, Differential effects of pertussis toxin and lithium on muscarinic response in the atria and ileum: evidence for receptor heterogeneity, Br. J. Pharmacol. 91, 6.

Ewald, D.A., I.-H. Pang and P.C. Sternweis, 1989, Differential G protein-mediated coupling of neurotransmitter receptors to Ca^{2+} channels in rat dorsal root ganglion neurons in vitro, Neuron 2, 1185.

Farmer, S.G. and R.M. Burch, 1992, Biochemical and molecular pharmacology of kinin receptors, Annu. Rev. Pharmacol. Toxicol. 32, 511.

Giannini, G., E. Clemment and R. Ceci, 1992, Expression of a ryanodine receptor-Ca^{2+} channel that is regulated by TGF-β, Science 257, 91.

Gil-Longo, J., M.N. Dufour, G. Guillon and C. Lugnier, 1993, G proteins in aortic endothelial cells and bradykinin-induced formation of nitric oxide, Eur. J. Pharmacol. 247, 119.

Guimarães, C.L., J.B. Calixto and G.A. Rae, 1992, Potent constrictor actions of endothelin-1, endothelin-2 and endothelin-3 in rat isolated portal vein, Hypertension 19, II-80.

Hall, J.M., 1992, Bradykinin receptors: pharmacological properties and biological roles, Pharmacol. Ther. 56, 131.

Hall, J.M., M.P. Caulfield, S.P. Watson and S. Guard, 1993, Receptor subtypes or species homologues. Relevance to drug discovery, Trends Pharmacol. Sci. 14, 376.

Hess, J.F., J.A. Borkowski, G.S. Young, C.D. Strader and R.W. Ransom, 1992, Cloning and pharmacological characterization of a human bradykinin (BK_2) receptor, Biochem. Biophys. Res. Commun. 184, 260.

Hess, J.F., J.A. Borkowski, T. Macneil, G.Y. Stonesifer, J. Fraher, C.D. Strader and R.W. Ransom, 1994, Differential pharmacology of cloned human and mouse B_2 bradykinin receptors, Mol. Pharmacol. 45, 1.

Hidaka, H. and R. Kobayashi, 1992, Pharmacology of protein kinase C inhibitors, Annu. Rev. Pharmacol. Toxicol. 32, 377.

Kremer, S., P. Harper, R. Hegele and K. Skorecki, 1987, Bradykinin stimulates a rise in cytosolic calcium in renal glomerular mesangial cells via a pertussis toxin insensitive pathway, Can. J. Physiol. Pharmacol. 66, 43.

Liao, J.K. and C.J. Homcy, 1994, The G-proteins of the G alpha (i) and G alpha (q) family couple the bradykinin receptor to the release of endothelium-derived relaxing factor, J. Clin. Invest. 92, 2168.

Liebmann, C., S. Orffermanns, K. Spicherk, K.-D. Hinsch, M. Schmittler, J.M. Morgat, S. Reissmann, G. Schutz and W. Rosenthal, 1990, A high-affinity bradykinin receptor in membranes from rat myometrium is coupled to pertussis toxin-sensitive G proteins of the G_i family, Biochem. Biophys. Res. Commun. 167, 910.

McEachern, A.E., E.R. Shelton, S. Bhakta, R. Overnolte, C. Bach, P. Zuppan, J. Fujisaki, R.W. Aldrich and K. Jarnigan, 1991, Expression cloning of a rat B_2 bradykinin receptor, Proc. Natl. Acad. Sci. USA 88, 7724.

McPherson, P.S. and K.P. Campbell, 1993, The ryanodine receptor/Ca^{2+} release channel, J. Biol. Chem. 268, 13765.

Medeiros, Y.S. and J.B. Calixto, 1993, Analysis of the mechanisms underlying the biphasic responses to bradykinin in circular muscle from guinea pig ileum, Eur. J. Pharmacol. 241, 157.

Meissner, G., 1994, Ryanodine receptor Ca^{2+} release channels and their regulation by endogenous effectors, Annu. Rev. Physiol. 56, 485.

Milligan, G., Z. Tanfin, O. Goureau, C. Unson and S. Harbon, 1989, Identification of both G_{i2} and novel, immunologically distinct forms of G_o in rat myometrial membranes, FEBS Lett. 244, 411.

Murayama, T. and M. Ui, 1983, Loss of the inhibitory function of guanine nucleotide regulatory component of adenylate cyclase due to its ADP ribosylation by silet activating protein, pertussis toxin, in adipocyte membranes, J. Biol. Chem. 258, 3319.

Perney, T.M. and R.J. Miller, 1989, Two different G-proteins mediated neuropeptide Y and bradykinin-stimulated phospholipid breakdown in cultured rat sensory neurons, J. Biol. Chem. 264, 7371.

Rasmussen, H., Y. Takuwa and S. Parks, 1987, Protein kinase C in the regulation of smooth muscle contraction, Fed. Am. Soc. Exp. Biol. J. 1, 177.

Regoli, D. and J. Barabé, 1980, Pharmacology of bradykinin and related kinins, Pharmacol. Rev. 32, 1.

Ruegg, U.T. and G.M. Burguess, 1989, Staurosporine K-252 and UCN-01: potent but non-specific inhibitors of protein kinase C, Trends Pharmacol. Sci. 10, 218.

Schlemper, V. and J.B. Calixto, 1994, Nitric oxide pathway-mediated relaxant effect of bradykinin in the guinea pig isolated trachea, Br. J. Pharmacol. 111, 83.

Shimamoto, Y., H. Shimamoto, C.Y. Kwan and E.E. Daniel, 1993, Differential effects of putative protein kinase C inhibitors on contraction of rat aortic smooth muscle, Am. J. Physiol. 264, H1300.

Sorrentino, V. and P. Volpe, 1993, Ryanodine receptors: how many, where and why?, Trends Pharmacol. Sci. 14, 98.

Tanfin, Z. and S. Harbon, 1987, Heterologous regulation of G proteins in rat myometrium. A differential modulation of $Gi_{3\alpha}$ during gestation, Mol. Pharmacol. 32, 249.

Tanfin, Z., O. Goureau, G. Milligan and S. Harbon, 1991, Characterization of G proteins in rat myometrium: a differential modulation of $Gi_{2\alpha}$ and $Gi_{3\alpha}$ during gestation, FEBS Lett. 278, 4.

Ui, M., 1986, Pertussis toxin as a toxin of receptor coupling to inositol lipid metabolism, in: Phosphoinositides and Receptor Mechanism (Alan R. Liss, New York) p. 163.

Wilkb-Laszczak, M.A., S. Gutowski, P.C. Sternweis and F. Belardetti, 1994, Bradykinin modulates potassium and calcium currents in neuroblastoma hybrid cells via different pertussis toxin insensitive pathways, Neuron 12, 109.

Wilkinson, S.E., P.J. Parker and J.S. Nixon, 1993, Isoenzyme specificity of bisindolymaleimides, selective inhibitors of protein kinase C, Biochem. J. 2, 335.

Yang, C.M., H.-C. Hsia, S.-F. Luo, J.-T. Hsieh and R. Ong, 1994, The effect of cyclic AMP elevating agents on bradykinin- and carbachol-induced signal transduction in canine cultured tracheal smooth muscle cells, Br. J. Pharmacol. 112, 781.