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Study of the mechanisms involved in the bradykinin-induced contraction of the pig iris sphincter muscle in vitro

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Abstract

This study was designed to investigate the mechanisms by which bradykinin induces contraction of the pig iris sphincter muscle in vitro. Addition of bradykinin, Lys-bradykinin and Met-Lys-bradykinin to the pig iris sphincter resulted in a graded contraction with a mean EC_{50s} of 21, 11 and 5 nM, respectively. The bradykinin B_1 receptor agonist des-Arg^9-bradykinin only caused a slight contraction, measured 6 h after the tissue was set up. The B_2 receptor antagonists FR 173657 ((*E*)-3-(6-acetamido-3-pyridyl)-*N* [*N*-2-4-dichloro-3-[(2-methyl-8-quinolinyl) oxymethyl] phenyl]-*N*-methylamino-carbonyl-ethyl] acrylamide) and Hoe 140 (D-Arg^0-[Hyp^3, Thi^5, D-Tic^7, Oic^8]-bradykinin produced a graded shift to the right associated with marked inhibition of the bradykinin-induced contraction. Atropine, guanethidine or tetrodotoxin significantly reduced the bradykinin-induced contraction. Dazoxiben, an inhibitor of thromboxane A_2, and MK-571 (3-(3-(2-(7-chloro-2-quinolinyl) ethenyl) phenyl ((3-dimethyl amino-3oxo-propyl) thio) methyl) propanoic acid, a leukotriene D_4 receptor-selective antagonist, also caused inhibition of the bradykinin-mediated contraction. Cyclooxygenase-1 and -2 inhibitors, indomethacin, ibuprofen, valeryl salicylate and NS 398 (*N*-[2-(cyclohexyloxy)-4-nitrophenyl]methanosulfonamide) all significantly inhibited the bradykinin-mediated contraction without affecting the carbachol-induced contraction of the pig iris sphincter. Taken together, these results indicate that the bradykinin-mediated contraction of the pig iris sphincter muscle seems to be mediated primarily by the activation of the B_2 receptor release of acetylcholine, noradrenaline and both cyclooxygenase-1 and -2 metabolites besides the release of leukotriene D_4 and tromboxane A_2 from the arachidonic acid pathway.

Keywords: Bradykinin; Iris sphincter, pig; Contraction; Cyclooxygenase metabolite; Bradykinin B_1 receptor-selective antagonist; Bradykinin B_2 receptor-selective antagonist

1. Introduction

Bradykinin is a nonapeptide that is generated in plasma and in most tissues from α_2-globulin kininogen by the action of the enzyme kallikrein, following tissue damage or infection. Acting as a local hormone, bradykinin binds to specific membrane receptors and participates in several pathophysiological states. Kinins are potent vasoactive peptides and promote venular dilation, increase vascular permeability, cause pain and hyperalgesia and are implicated in several inflammatory states (see for review Regoli and Barabé, 1980; Farmer and Burch, 1992; Calixto et al., 2000, 2001).

The actions of kinins are mediated by the activation of two types of membrane receptors, B_1 and B_2. The bradykinin B_1 receptors are rarely expressed under normal conditions but they are up-regulated in certain pathological states, after tissue injury, or during in vitro incubation. The bradykinin B_1 receptors exhibit greater affinity for the kinin metabolites des-Arg^9-bradykinin and des-Arg^{10}-kallidin (Lys-des-Arg^9-bradykinin) than for bradykinin itself, and they are blocked by selective bradykinin B_1 receptor antagonists. In contrast, the B_2 receptors for kinins are widely distributed throughout the peripheral and central nervous systems, show high affinity for bradykinin and lysyl bradykinin (Lys-bradykinin) and seem to mediate most of the actions of kinins under normal conditions (Farmer and Burch, 1992; Hall, 1992). Bradykinin acting through B_2

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receptors exerts inflammatory actions in the rabbit eye and induces contraction in the rabbit iris, an effect which is generally considered to be largely dependent on the release of sensory neuropeptides, particularly tachykinin from capsaicin-sensitive fibres of trigeminal origin (Cole and Unger, 1974; Zhang et al., 1982; Ueda et al., 1982; Wahlestedt et al., 1985; Wang and Hakanson, 1993; Hall et al., 1995). Geppetti et al. (1990) have shown that bradykinin caused concentration-dependent contraction of pig iris sphincter muscle in vitro through a mechanism that is largely independent of neuropeptide release from capsaicin-sensitive sensory neurones.

In the present study, we sought to investigate the receptor subtype and also some of the mechanisms by which bradykinin induces contraction of the pig iris sphincter muscle in vitro.

2. Methods

2.1. Tissue preparation

Pig eyes were obtained from a local slaughterhouse and were immediately transported on ice to the laboratory. Tissues were used within 6 h after death. The iris was rapidly dissected and placed in a Petri dish containing warmed Krebs–Henseileit solution (see composition below) oxygenated with 95% of O_2 and 5% of CO_2, and maintained at 37 °C. The irises were carefully dissected from adherent tissues (two preparations per eye). Each iris was mounted in a 5-ml organ chamber containing Krebs–Henseleit solution, maintained at 37 °C, pH 7.4. The Krebs solution had the following composition (in mmol/l): NaCl, 118.0; KCl, 4.4; $CaCl_2$, 2.5; $NaHCO_3$, 25.0; $MgSO_4$, 1.1; KH_2PO_4, 1.2; glucose, 11.0. The preparation was connected vertically to a force-displacement transducer under a resting tension of 100 mg. The optimal tension was adjusted on the basis of preliminary experiments in which different resting tensions (50–300 mg) were assessed. The resting tension of 100 mg showed the best response and was selected for further experiments. Preparations were allowed to equilibrate for at least 90 min before drug addition, during which the buffer solution was refreshed every 15 min. Isometric contractions were recorded by means of a polygraph (TRI-201 Letica Scientific Instruments, Spain). The contractile responses to bradykinin and the other agonists are expressed as percentages of the contraction induced by 80 mmol of KCl. Usually, four to six preparations (two obtained from each eye) were mounted in parallel. In all experiments, at least one preparation received the agonist alone plus vehicle and served as control.

2.2. Contraction induced by bradykinin and other agonists

After a stabilisation period of at least 90 min, and in order to confirm the viability of the tissues, preparations were exposed to a high-potassium concentration (KCl 80 mmol, prepared by equimolar replacement of 74.4 mmol of NaCl by KCl) as a standard stimulus. After washout replacement with normal medium and return to the original baseline, complete concentration-response curves were obtained at 60-min intervals for bradykinin (0.1–10.000 nM) and related kinins (Met-Lys-bradykinin, Lys-bradykinin, 0.1–10.000 nM) in the presence or in the absence of the protease inhibitor captopril (3 μM). The concentration-response curves for all studied agonists were made by means of the cumulative method (Van Rossum, 1963). Each concentration of the agonist was added to the bath when the effect of the preceding addition had reached its maximum. No significant desensitisation was observed for at least three consecutive concentration-response curves for bradykinin and related kinins performed in the same preparation. Accordingly, no more than three complete curves were recorded for each tissue.

In another set of experiments, after stable concentration-response curves for bradykinin were obtained, preparations were pre-incubated with a selective bradykinin B_2 receptor antagonist Hoe 140 (D-Arg^0-[Hyp^3, Thi^5, D-Tic^7, Oic^8]-BK (icatibant) (0.1–3 pM) or FR 173657 ((*E*)-3-(6-acetamido-3-pyridyl)-*N* [*N*-2-4-dichloro-3-[(2-methyl-8-quinolinyl) oxymethyl]phenyl]-*N*-methylamino-carbonyl-ethyl] acrylamide) (10–100 nM) or with the selective B_1 receptor antagonist des-Arg^9-[Leu^8]-bradykinin (1 μM). In a separate series of experiments, different intervals of contact antagonists (5–30 min) with preparations were tested. The results demonstrated that a 10-min interval proved to be ideal and was selected for future experiments. Accordingly, all antagonists were added to the preparations at least 10 min before challenge with the agonist. Control experiments for bradykinin were always carried out in parallel in the presence of phosphate-buffered saline solution (concentration: NaCl, 137 mM; KCl, 2.7 mM; phosphate buffer, 10 mM) in order to correct for any time-dependent changes in the responsiveness of the preparations to bradykinin.

The possible contribution of the release of neurotransmitters to the bradykinin-mediated contraction of the pig iris sphincter muscle was also investigated. To this end, preparations were treated with one of the following agents: atropine (an anticholinergic agent, 1 μM), guanethidine (a norepinephrine depletor, 1 μM) or tetrodotoxin (a Na^+ channel blocker, 1 μM). All drugs were pre-incubated with the tissues 20 min beforehand. Only one antagonist was used in each preparation.

To assess the possible involvement of arachidonic acid metabolites derived from both cyclooxygenase-1 and -2 in the bradykinin-induced contractile response of the pig iris sphincter, preparations were pre-incubated for 20 min with one of the following drugs: indomethacin (1 μM) or ibuprofen (10 μM) (non-selective cyclooxygenase-1 and -2 inhibitors), valeryl salicylate (10 μM, a preferential cyclooxygenase-1 inhibitor) or NS 398 (*N*-[2-(cyclohexyloxy)-4-nitrophenyl] methanosulfonamide), (10 μM, a

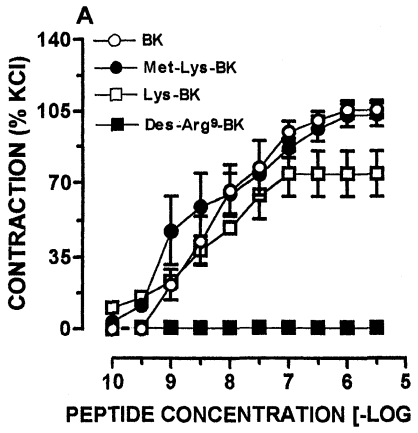

Fig. 1. Cumulative log concentration-response curves for (A) BK, Met-Lys-BK, Lys-BK and des-Arg^9-BK in the isolated pig iris. Results are expressed as percentages of the contraction induced by 80 mmol of KCl. Each point represents the mean, with vertical lines showing S.E.M. for five experiments. Significant differences from respective control values where $^*P < 0.05$ (Student's unpaired *t*-test).

preferential cyclooxygenase-2 inhibitor)—and new concentration-response curves for bradykinin were obtained in their presence. To examine the role of thromboxane A_2 and leukotriene in the bradykinin-mediated contraction of the pig iris sphincter, preparations were pre-incubated with dazoxiben (100 nM, an inhibitor of thromboxane A_2-synthase or with MK-571 (3-(3-(2-(7-chloro-2-quinolinyl) ethenyl) phenyl ((3-dimethyl amino-3oxo-propyl) thio) methyl)) propanoic acid (100 nM, a leukotriene D_4 selective receptor antagonist) and complete concentration-response curves were obtained for bradykinin in their presence.

2.3. Statistical analysis

All values are expressed as the mean ± S.E.M., except the EC_{50} values (i.e. the molar concentration of the agonists required to produce 50% of the maximal response), which are reported as the geometric means accompanied by 95% confidence limits. The EC_{50} values were calculated by means of linear regression analysis from complete concentration-response curves in individual experiments. Tests for statistical significance were performed using Student's *t*-test, either paired or unpaired. $P < 0.05$ or less was considered as indicative of significance.

2.4. Drugs

The following drugs were used: Bradykinin, Met-lys-bradykinin, Lys-bradykinin, des-Arg^9-bradykinin, des-Arg^9-[Leu^8]bradykinin, tetrodotoxin, guanethidine, indomethacin, ibuprofen, carbachol (all from Sigma, St. Louis, MO, USA), NS 398 (*N*-[2-(cyclohexyloxy)-4-nitrophenyl]methanosulfonamide), valeryl (pentanoyl) salicylate (Cayman Chemical, Ann Arbor, MI, USA), captopril (Research Biochemicals International, Natick, MA, USA), dazoxiben (Pfizer, Kent, UK), atropine sulphate, MK-571 (3-(3-(2-(7-chloro-2-quinolinyl) ethenyl) phenyl ((3-dimethyl amino-3oxo-propyl) thio) methyl)) propanoic acid (E. Merck, Darmstadt, Germany) and the bradykinin B_2 receptor antagonists FR 173657 ((*E*)-3-(6-acetamido-3-pyridyl)-*N* [*N*-2-4-dichloro-3-[(2-methyl-8-quinolinyl) oxymethyl] phenyl]-*N*-methylamino-carbonyl-ethyl] acrylamide) (Fujisawa, Osaka, Japan) and Hoe 140 (D-Arg^0-[Hyp^3, Thi^5, D-Tic^7, Oic^8]-bradykinin (Aventis Pharma Deutschland, Frankfurt Main, Germany).

The stock solutions for all peptides used were prepared in phosphate-buffered saline (PBS; 1–100 mM), kept in siliconized plastic tubes and stored in a freezer at −18 °C until use. Stock solutions of indomethacin were made in absolute ethanol. Valeryl salicylate and NS 398 were dissolved in dimethyl sulfoxide. All other drugs were dissolved in phosphate-buffered saline solution to the desired concentration just before use. The final bath concentration of ethanol and dimethyl sulfoxide did not exceed 0.02%, which alone had no effect on the tone of the preparations or on the bradykinin-mediated contraction.

In all experiments, to correct for any possible time-dependent changes in the responsiveness of the preparations, at least one parallel control preparation was exposed to the vehicle used to dissolve each drug, and this was used as control.

3. Results

Fig. 1A shows that cumulative addition of bradykinin to the pig iris sphincter muscle produced a concentration-dependent and well-reproducible contractile response with no evidence of tachyphylaxis. Pre-incubation of the preparations with captopril (3 μM) did not significantly affect the bradykinin-mediated contraction, either at the level of the EC_{50} or at the level of the maximal developed response (results not shown). Addition of the kinin agonists Lys-bradykinin and Met-Lys-bradykinin to the bath produced a concentration-dependent contraction of the pig iris sphincter (Fig. 1A). At the EC_{50} level, all kinin agonists had very similar effects (Table 1), and compared with KCl-induced

Table 1
Potency and maximal contractile responses induced by bradykinin and related peptides in the pig iris in vitro

Kinin agonist	EC_{50} (95% confidence limits) (nM)	Maximal response (% KCl 80 mM)
Met-Lys-BK	4.7 (1.4–15.0)	103 ± 5
Lys-BK	11.0 (6.0–30.0)	74 ± 11
BK	21.0 (12.0–47.0)	106 ± 4

Each group represents the mean of four to six experiments.

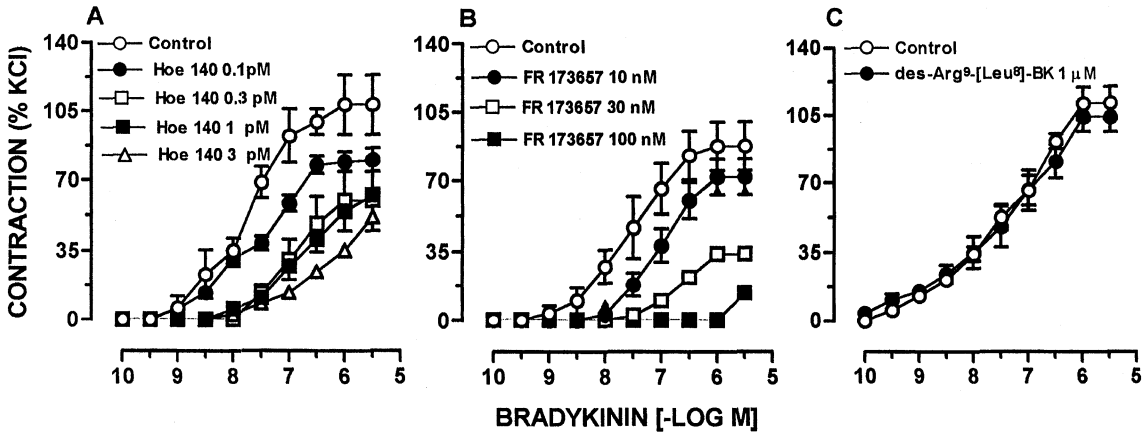

Fig. 2. Cumulative log concentration-response curves for BK in the isolated pig iris obtained in the absence or presence of (A) Hoe 140 (0.1, 0.3, 1 and 3 pM); (B) FR 173657 (10, 30 and 100 nM); or (C) des-Arg^9[Leu^8]BK (1 μM). Results are expressed as percentages of the contraction induced by 80 mmol of KCl. Each point represents the mean, with vertical lines showing S.E.M. for five experiments. In some points, the error deviation is hidden inside the symbol. Significant differences from respective control values where $*P<0.05$ (Student's unpaired *t*-test).

contraction, the efficacy of the kinin agonists did not differ significantly (Fig. 1A and Table 1). However, addition of the selective bradykinin B_1 receptor agonist des-Arg^9-bradykinin (up to 1 μM) 90 min after the preparations were set up did not cause any contractile response in the pig iris sphincter (Fig. 1A). In preparations that remained in equilibration for up to 6 h, the bradykinin B_1 receptor agonist des-Arg^9-bradykinin produced a weak contraction (15% relative to that caused by KCl) (results not shown).

Pre-incubation of the preparations with des-Arg^9[Leu^8]-bradykinin (1 μM), a selective B_1 receptor antagonist, had no significant effect on the preparation baseline and failed to affect the bradykinin-induced contraction of the pig iris sphincter (Fig. 2C). The results of Fig. 2A and B show that, in contrast, pre-incubation of the preparations with the selective kinin B_2 receptor antagonists Hoe 140 (0.1–3 pM) or FR 173657 (10–100 nM) caused a concentration-dependent shift to the right associated with a marked inhibition of the bradykinin concentration-response curves in the pig iris sphincter.

Fig. 3A and B shows that pre-incubation of the pig iris sphincter muscle with atropine (1 μM) 20 min beforehand caused a 6.6-fold shift to the right of the bradykinin-induced contraction, while guanethidine (1 μM) produced a marked inhibition of the bradykinin-mediated contraction (60 ± 5%). Furthermore, addition of tetrodotoxin (1 μM) to the preparations significantly reduced (31 ± 3%) the contraction induced by bradykinin (Fig. 3C). Pre-incubation of the preparations with indomethacin (1 μM), ibuprofen (10 μM), valeryl salicylate (10 μM) or NS 398 (10 μM) also significantly inhibited the bradykinin-mediated contraction (52 ± 5%, 47 ± 6%, 33 ± 5% and 53 ± 8%, respectively) (Fig. 4A–D). The addition of dazoxiben or MK-571 (both 100 nM) to the preparations 30 min beforehand also produced a significant inhibition (23 ± 4% and 33 ± 6%, respectively) (Fig. 4E–F). At the same concentrations, indomethacin, ibuprofen,

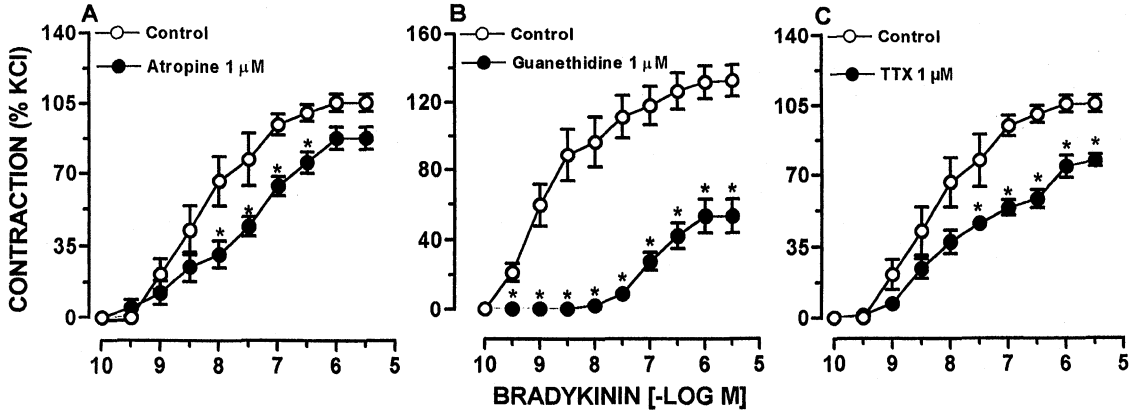

Fig. 3. Cumulative log concentration-response curves obtained for BK in the isolated pig iris in the absence or presence of (A) atropine (1 μM), (B) guanethidine (1 μM) and (C) tetrodotoxin (TTX) (1 μM). Results are expressed as percentages of the contraction induced by 80 mmol of KCl. Each point represents the mean, with vertical lines showing S.E.M. for five experiments. Significant differences from respective control values where $*P<0.05$ (Student's unpaired *t*-test).

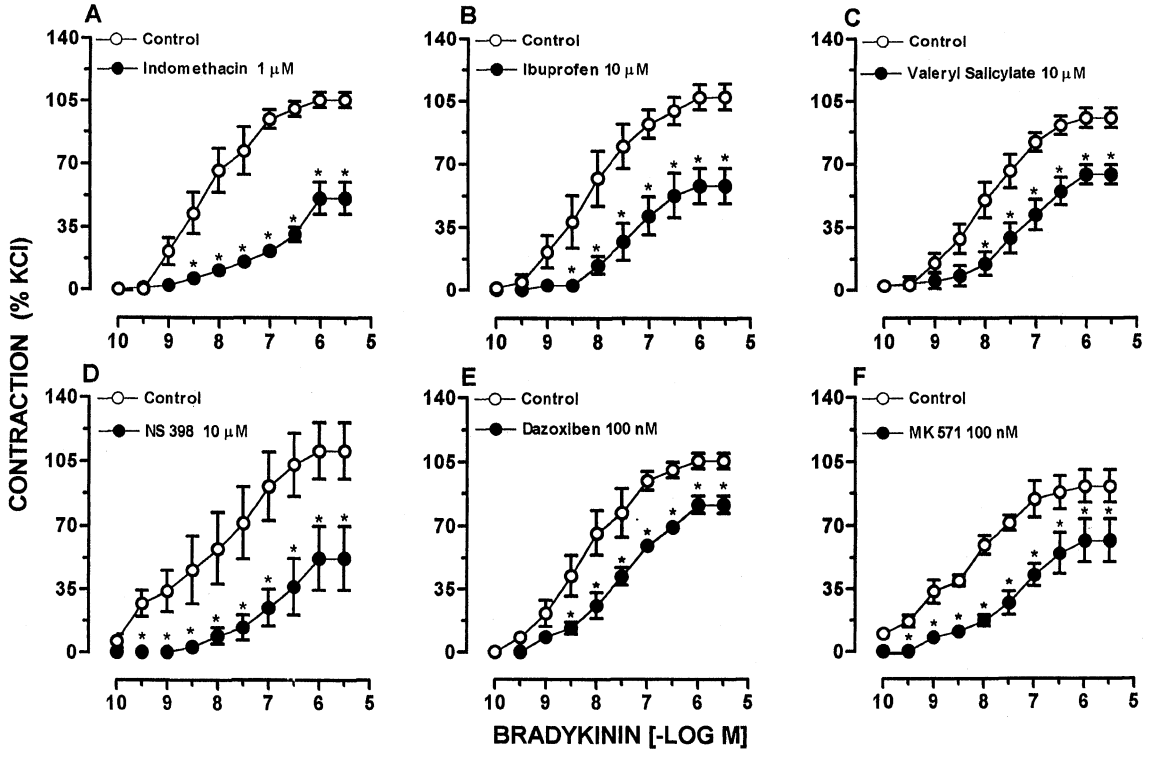

Fig. 4. Cumulative log concentration-response curves obtained for BK in the isolated pig iris in the absence or presence of (A) indomethacin (1 μM), (B) ibuprofen (10 μM), (C) valeryl salicylate (10 μM), (D) NS 398 (10 μM), (E) dazoxiben (100 nM) and (F) MK-571 (100 nM). Results are expressed as percentages of the contraction induced by 80 mmol of KCl. Each point represents the mean, with vertical lines showing S.E.M. for five experiments. In some points, the error deviation is hidden inside the symbol. Significant differences from respective control values where $*P<0.05$ (Student's unpaired *t*-test).

valeryl salicitate, NS 398, dazoxiben or MK-571 all failed to significantly affect the contractile response caused by carbachol (0.01–10 μM) in pig iris sphincter (results not shown).

4. Discussion

The results of the present study show that bradykinin and related peptides (Lys-bradykinin, Met-Lys-bradykinin) produce potent, concentration-dependent and well-reproducible contractile responses of the sphincter muscle of the pig iris in vitro. By using selective agonists and antagonists of kinin receptors, it was possible to demonstrate pharmacologically that the activation of the kinin B_2 receptor subtype is mainly, if not solely, responsible for mediating the contractile response to kinin of the pig iris sphincter muscle. This observation derives from the view that the selective B_2 receptor antagonists Hoe 140 and FR 173657 concentration dependently and potently antagonized the bradykinin-mediated contraction in these preparations under conditions where the bradykinin B_1 receptor selective antagonist des-Arg^9[Leu^8]bradykinin had no significant effect on the bradykinin-mediated contraction. An additional piece of evidence supporting this view was the observation that the selective bradykinin B_1 receptor agonist des-Arg^9-bradykinin, even at higher concentrations (up to 1 μM), had no detectable effect on the pig iris sphincter muscle. However, when assessed 6 h after the set up of the preparations, a small contraction (15% relative to that caused by KCl) was evoked by the selective bradykinin B_1 receptor agonist des-Arg^9-bradykinin. These results suggest that the B_1 receptor can be up-regulated in the pig iris sphincter muscle in vitro. The existence of an inducible bradykinin B_1 receptor mediating the contraction elicited by des-Arg^9-bradykinin, which is sensitive to cycloheximide, has been previously reported for porcine iliac arteries (Persson and Andersson, 1998).

Concerning the mechanism which underlies the bradykinin-mediated contraction of the pig iris sphincter muscle, the present result shows that both selective peptide and non-peptide bradykinin B_2 receptor antagonists (Hoe 140 and FR 173657, respectively) behave as non-competitive antagonists of the bradykinin response. Data reported in the literature show that in most tissues, e.g. mouse and rat vas deferens, rat stomach and portal vein, guinea pig gallbladder and ileum (Maas et al., 1995; Asghar et al., 1993; Falcone et al., 1993; Cabrini et al., 1995; Medeiros and Calixto, 1993; Hock et al., 1991), Hoe 140 and FR 173657 work as competitive bradykinin B_2 receptor antagonists. However, in other smooth muscle preparations, Hoe 140 acts through a mixture of competitive and non-competitive mechanisms on bradykinin-mediated contraction (Rhaleb et al., 1992; Griesbacher and Lembeck, 1992; Field et al., 1992; Trifilieff et al., 1992; Cabrini et al., 1996). Likewise, the non-peptide

and selective bradykinin B_2 receptor antagonist FR 173657 behaves as a competitive or mixed competitive and non-competitive antagonist depending on the smooth muscle tissue studied (Griesbacher et al., 1997). Together, these findings further support the existence of a great variation in bradykinin receptors among animal species and/or tissues (Hall, 1992).

The lack of effect of captopril in potentiating the bradykinin-induced contraction of the pig iris sphincter muscle strongly suggests the absence of the enzyme kininase II in this tissue. These findings are in perfect agreement with those previously reported by Igic (1985), who showed that the activity of kininase II varies considerably in pig ocular tissues. Thus, while the activity of kininase II is higher in choroid, and to a lesser extent in the retina and ciliary body, only traces of the enzyme are found in the iris. Similar results have been reported for bradykinin in the rabbit iris (Wang and Hakanson, 1993). Furthermore, kininase I has also been found in pig ocular tissues, having higher activity in the aqueous humor, serum, retina, choroid and cilium, and lower activity in the iris (Igic, 1985).

A great amount of in vitro and in vivo evidence now suggests that the actions of bradykinin in most tissues are indirectly mediated via the release of several mediators, such as prostanoids and nitric oxide (Vianna and Calixto, 1998; Geppetti, 1993; Campos and Calixto, 2000). Thus, we sought to examine in the current study whether or not the bradykinin-mediated contraction of the pig iris is also mediated by the release of the above-mentioned mediators. Our results show that the contraction caused by bradykinin of the pig iris sphincter muscle is largely dependent on the neural release of acetylcholine, because atropine markedly antagonized the bradykinin-mediated contraction. Furthermore, tetrodotoxin largely antagonized the bradykinin-induced contraction of the pig iris sphincter muscle, further confirming an indirect mediation and the involvement of a neural mechanism of activation by the peptide. An interesting result was the observation that guanethidine, a norepinephrine depletor, markedly reduced the maximal bradykinin-developed tension associated with a pronounced shift to the right of the peptide concentration-response curves for the pig iris. Such results provide evidence that the release of the adrenergic neurotransmitter norepinephrine seems to play a critical role in the bradykinin-mediated contraction of this preparation. It is well known that α_1- and α_2-adrenoceptors exist in the eye. The presence of the α_2-adrenoceptor subtypes has been investigated using radioligand binding techniques in four different parts of the pig eye, namely the choroid, ciliary body, iris and retina. The results indicated that the only α_2-adrenoceptor subtype present in the pig choroid, ciliary body and iris is the α_{2A} receptor subtype, while the retina contains both α_{2A}- and α_{2c}-adrenoceptors (Matsson-Wikberg et al., 1996). Matsson-Wikberg et al. (2000), using binding techniques, also demonstrated the presence of both α_{1A}- and α_{1B}-adrenoceptors in the pig retina.

Furthermore, our results also show that prostanoids derived from both cyclooxygenase-1 and -2 pathways of arachidonic acid metabolism mediate the contractile response elicited by bradykinin in the pig iris. Such a notion derives from the view that the non-selective cyclooxygenase-1 and -2 antagonists (indomethacin, ibuprofen) and also the preferential cyclooxygenase-1 (valeryl salicylate) and -2 (NS 398) inhibition, each at a concentration known to inhibit such enzymes, consistently prevented the bradykinin mediated contraction of the pig iris sphincter muscle. Our data also suggest that thromboxane A_2 mediates the bradykinin-evoked contraction of the pig iris, because dazoxiben significantly antagonized the bradykinin response. In addition, the lipoxygenase metabolite derived from arachidonic acid and leukotriene D_4 also mediates the bradykinin-evoked contraction of pig iris because the selective leukotriene D_4 receptor antagonist MK-571 significantly prevented the bradykinin mediated contraction. Likewise, Turner et al. (2000) have reported that the bronchoconstriction elicited by bradykinin in guinea pigs is mediated by the release of leukotriene D_4. To exclude non-specific action of the used inhibitors, we tested the same concentration of them against the carbachol-mediated contraction of the pig iris. Our results showed that at the same concentration at which they markedly affected the bradykinin-mediated contraction of the pig iris, they failed to affect the carbachol-mediated responses, indicating their selectivity of action.

In conclusion, the results of the present study demonstrate that bradykinin and related peptides induce potent and reproducible concentration dependent contractions of the pig iris sphincter muscle in vitro through the activation of bradykinin B_2 receptors. The possible presence of inducible bradykinin B_1 receptors was only observed to a limited extent 6 h after the preparations were set up. Furthermore, our results show that the bradykinin-mediated contraction in these preparations is in great part mediated by the neural release of acetylcholine and noradrenaline, since bradykinin-mediated contractions of the pig iris were prevented by atropine, guanethidine or tetrodotoxin. Finally, the bradykinin-mediated contraction of the pig iris involves the release of prostanoids derived from both cyclooxygenase-1 and -2 and also the release of leukotriene D_4 and tromboxane A_2 from arachidonic acid pathways.

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