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Characterization of kinin receptors modulating neurogenic contractions of the mouse isolated vas deferens

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1 This study analyses the receptors mediating the effects of bradykinin (BK) and analogues on neurogenic twitch contractions of the mouse isolated vas deferens evoked, in the presence of captopril (3 μM), by electrical field stimulation with trains of 4 rectangular 0.5 ms pulses of supramaximal strength, delivered at a frequency of 10 Hz every 20 s.

2 BK (0.1–300 nM) induced a graded potentiation of twitches, with an EC_{50} (geometric mean and 95% confidence limits) of 4.5 nM (1.7–11.6) and an E_{max} of 315 ± 19 mg per 10 mg of wet tissue ($n = 6$). Similar results were obtained in tissues challenged with Lys-BK, [Hyp^3]-BK, Met,Lys-BK and the selective B_2 receptor agonist [Tyr(Me)^8]-BK (0.1–300 nM).

3 The selective B_2 receptor antagonists, Hoe 140 (1-10 nM) and NPC 17731 (3–30 nM), caused graded rightward shifts of the curve to BK-induced twitch potentiation, yielding apparent pA_2 values of 9.65 ± 0.09 and 9.08 ± 0.13, respectively, and Schild plot slopes not different from 1. Both antagonists (100 nM) failed to modify similar twitch potentiations induced by substance P (3 nM) or endothelin-1 (1 nM). Preincubation with the selective B_1 receptor antagonist, [Leu^8,des-Arg^9]-BK (1 μM), increased the potentiating effect of BK on twitches at 30–300 nM.

4 In contrast to BK, the selective B_1 receptor agonist, [des-Arg^9]-BK (0.3–1000 nM) reduced the amplitude of twitches in a graded fashion, with an IC_{50} of 13.7 nM (10.4–16.1) and an I_{max} of 175 ± 11 mg ($n = 4$). The twitch depression induced by [des-Arg^9]-BK (300 nM) was not affected by Hoe 140 (30 nM) or NPC 17731 (100 nM), but was abolished by the selective B_1 receptor antagonist, [Leu^8,des-Arg^9]-BK (1 μM), which did not modify the twitch inhibitory effect of clonidine (1 nM) or morphine (300 nM).

5 In non-stimulated preparations, BK (100 nM) also potentiated, in a Hoe 140-sensitive (10 nM) manner, the contractions induced by ATP (100 μM), but not by noradrenaline (10 μM), whereas [des-Arg^9]-BK (300 nM) did not modify the contractions induced by either agonist.

6 It is concluded that the mouse vas deferens expresses both B_1 and B_2 receptors, which modulate sympathetic neurotransmission in opposing ways. Neurogenic contractions are inhibited by stimulation of possibly prejunctional B_1 receptors, whereas activation of B_2 receptors increases twitch contractions, in part by amplifying the responsiveness of the smooth muscle cells to the sympathetic co-transmitter ATP.

Keywords: Bradykinin; B_1 receptors; B_2 receptors; mouse vas deferens; neurotransmission; bradykinin antagonist; Hoe 140; NPC 17731

Introduction

In addition to its widespread biological effects (for reviews see Bhoola *et al.*, 1992; Hall, 1992), bradykinin (BK) can modify neurotransmission at certain autonomic neuroeffector junctions of the urogenital tract. Thus, BK potentiates neurogenic contractions of the rat isolated urinary bladder, possibly by increasing the responsiveness of smooth muscle cells to the sympathetic co-transmitter ATP (Acevedo *et al.*, 1990). Similarly, the peptide increases responses to electrical field stimulation of the vas deferens isolated from guinea-pigs (Zetler & Kampmann, 1979) or rats (Huidobro-Toro *et al.*, 1986; Tousignant *et al.*, 1987). In the rat vas deferens, BK also augments the release of [^{3}H]-noradrenaline, potentiates the contraction to ATP, but not noradrenaline, and causes smooth muscle contraction (Llona *et al.*, 1987; 1991; Donoso *et al.*, 1989). These effects appear to be mediated via stimulation of B_2 receptors (Llona *et al.*, 1987; Rifo *et al.*, 1987). Moreover, BK also triggers the release of adrenaline from adrenochromaffin cells and of noradrenaline from sympathetic ganglia (Collier, 1970).

Llona *et al.* (1991) reported that BK also enhances noradrenaline release from sympathetic nerves of the mouse vas deferens, but the effects of BK on neurotransmission in this preparation were not fully characterized. Sympathetic neurotransmission in the mouse vas deferens is accomplished via the release of the co-transmitters ATP, noradrenaline and, possibly at higher discharge frequencies, neuropeptide Y (Stjärne *et al.*, 1986; von Kügelgen *et al.*, 1989; Drake & Petersen, 1992). Neurogenic twitch contractions of this preparation can be modulated by a wide variety of mediators, being depressed by α_2-adrenoceptor agonists (Illes & Starke, 1983), purinoceptor agonists (Kurz *et al.*, 1993), opioids (Lord *et al.*, 1977), neuropeptide Y (Stjärne *et al.*, 1986) or calcitonin-gene-related peptide (Manzini & Parlani, 1992), while being increased by endothelins (Rae & Calixto, 1990) or tachykinins (Manzini & Parlani, 1992).

The aim of this study was to characterize, by the use of selective B_1 and B_2 receptor agonists and antagonists, the receptors mediating the effect of BK and related kinins on responses of the mouse vas deferens to electrical field stimulation. We have found that, unlike the vasa deferentia from other species, the mouse preparation displays both B_1 and B_2 receptors which modulate sympathetic neurotransmission in distinct ways.

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Methods

Tissue preparations

Experiments were conducted on male Swiss albino mice (25–35 g), raised in a temperature-controlled (22 ± 2°C) environment with 12 h light/dark cycle, and allowed free access to water and Purina lab chow. Animals were lightly anaesthetized with ether and killed by a sharp blow to the head and cervical dislocation. Both vasa deferentia were carefully excised, placed in a Petri dish containing warm physiological salt solution (see composition below) and freed of adhering connective and adipose tissues. Each vas deferens was set up in 5 ml organ baths containing Krebs solution at 37°C continuously gassed with 95% of O_2 and 5% of CO_2, connected to a strain gauge transducer coupled to a pen recorder (Narco Biosystems, U.S.A.) and submitted to an initial resting tension of 0.5 g. The Krebs solution had the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 25, KH_2PO_4 0.9 and glucose 11 (pH 7.2–7.4). Unless otherwise stated, the Krebs solution also contained captopril (3 μM) to prevent the action of kininase II. A stabilization period of at least 60 min was allowed before drug additions, during which the bath solution was renewed every 15 min. Electrical field stimulation was induced with trains of 4 rectangular 0.5 ms pulses of supramaximal strength (65–70 V) delivered, every 20 s, via a pair of platinum electrodes consisting of a hook below and a ring above each preparation.

Concentration-response curves to bradykinin and related peptides on twitch tension

Following stabilization of the twitch contractions evoked by electrical field stimulation, complete non-cumulative concentration-response curves were obtained to the twitch-potentiating effects of BK, Lys-BK, Met,Lys-BK, [Tyr(Me)^8]-BK or [Hyp^3]-BK, and to the twitch-depressor effect of [des-Arg^9]-BK (0.1 to 300 nM). Each agonist concentration was added to the medium for 2 min followed by 5 renewals of the bathing medium. Electrical field stimulation was interrupted just prior to washout of each agonist concentration and re-initiated 5 min before addition of the next agonist concentration.

Effects of B_1 and B_2 selective receptor antagonists on responses to bradykinin and des-Arg^9-bradykinin

Complete concentration-response curves were obtained for the twitch-potentiating effect of BK (0.1 to 300 nM) in the absence or in the presence of different concentrations of the selective B_2 receptor antagonists, Hoe 140 (1 to 10 nM) or NPC 17731 (3 to 30 nM), incubated with the tissues 5 min before each addition of BK. Only one complete concentration-response curve was carried out for BK in each preparation. Control experiments were carried out in the presence of the vehicle used to dilute the antagonists (phosphate buffer solution, PBS). The apparent pA_2 values (i.e. the co-logarithm of an antagonist's equilibrium dissociation constant or of its K_B) for Hoe 140 and NPC 17731 against BK-induced potentiation of neurogenic contractions were estimated by the rightward shifts they caused, at different concentrations, in the concentration-response curve to BK. The apparent pA_2 value for each antagonist was assessed from a Schild plot of log EC_{50} concentration-ratios minus 1 (CR − 1) versus log concentration of the antagonist, calculated by least square regression analysis (Arunlakshana & Schild, 1959). Antagonism was considered to be competitive in nature if the slope of the Schild regression line did not significantly differ from unity (Kenakin, 1993). Other experiments were performed to evaluate the influence of the selective B_1 receptor antagonist, [Leu^8,des-Arg^9]-BK on responsiveness to BK, in which 1 μM of the antagonist was added to the bathing medium 5 min before each addition of BK.

The effects of [Leu^8,des-Arg^9]-BK (1 μM), as well as those of Hoe 140 (100 nM) or NPC 17731 (100 nM) against the twitch-depressor response to [des-Arg^9]-BK (300 nM) were also assessed. Following a first control challenge with [des-Arg^9]-BK, each antagonist was added to the bathing medium for 5 min and a second challenge with the agonist conducted in its presence. The specificity of the actions of Hoe 140 and NPC 17731 against BK-induced responses was confirmed by testing their influence on twitch potentiations induced by equieffective concentrations of BK (300 nM), Lys-BK (300 nM), substance P (3 nM) or endothelin-1 (1 nM). The specificity of the action of [Leu^8,des-Arg^9]-BK was analysed by testing its influence against twitch inhibition by clonidine (1 nM) or morphine (300 nM). The preparations were usually challenged twice with a given agonist, first in the absence (control) and then in the presence of the antagonist, 30 min later. However, due to the very slow reversibility of endothelin-1-induced twitch potentiation in the mouse vas deferens (Rae & Calixto, 1990), both vasa deferentia from a given animal were each challenged only once with the agonist, one of them in the presence of the B_2 receptor antagonist.

Effects of bradykinin and [des-Arg^9]-bradykinin on contractions induced by noradrenaline and ATP

In other experiments, preparations not subjected to electrical field stimulation were challenged repeatedly, for 1 min at 30 min intervals, with ATP (100 μM) or noradrenaline (10 μM), at concentrations causing contractions similar in size to those evoked by electrical field stimulation. Once the responses stabilized (usually after 2–3 challenges), BK (100 nM) was added to the medium for 30–45 s and a new response to the agonist was obtained in its presence. A final challenge with ATP was also conducted in the presence of BK plus [Leu^8,des-Arg^9]-BK (1 μM), Hoe 140 or NPC 17731 (each at 100 nM), each added 5 min prior to BK. The effects of [des-Arg^9]-BK (300 nM) on responses to both ATP and noradrenaline were also tested. Only one contractile agonist, one kinin and one kinin receptor antagonist were tested in each preparation. All tissues were blotted and weighed after each experiment.

Statistical analysis

Agonist-induced changes in magnitude of twitch responses to electrical field stimulation are expressed as mean ± s.e.mean increases or decreases of twitch tension in mg per 10 mg of wet tissue, relative to the last basal twitch response prior to drug addition. E_{max} and I_{max} indicate maximal increase and maximal decrease of twitch tension induced by a given agonist, respectively. EC_{50} and IC_{50} values in individual experiments (i.e. the concentrations of agonist needed to cause half maximal potentiation or inhibition of twitch responses) were obtained by graphical interpolation and are presented as geometric means accompanied by their 95% confidence limits (Fleming *et al.*, 1972). The apparent pA_2 values are shown as mean ± s.e.mean and the slopes of the Schild plot regression lines as means accompanied by their 95% confidence limits (Kenakin, 1993). Statistical comparisons were performed by analysis of variance followed by Student's t test for paired or unpaired samples, where appropriate, and $P < 0.05$ was considered significant.

Drugs

Drugs used were: BK, Lys-BK, Met,Lys-BK, [Tyr(Me)^8]-BK, [Hyp^3]-BK, [Leu^8,des-Arg^9]-BK, substance P, captopril, clonidine hydrochloride, tetrodotoxin, guanethidine sulphate, ATP disodium, α,β-methylene ATP lithium, noradrenaline

bitartrate and prazosin hydrochloride (all from Sigma Chemical Company, St. Louis, U.S.A.), [des-Arg^9]-BK (Peninsula, Belmont, U.S.A.), endothelin-1 (Peptide Institute, Osaka, Japan), morphine hydrochloride (Merck A.G., Darmstadt, Germany). Hoe 140 (D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8-BK) and NPC 17731 {D-Arg-[Hyp^3,D-Hyp^E(transpropyl)^7,Oic^8]-BK} were kindly given by Hoechst A.G. (Frankfurt, Germany) and Scios/Nova (Baltimore, U.S.A.), respectively. Most stock solutions were made up in PBS, except those of prazosin and noradrenaline, which were made up in 100% ethanol and 0.1 N HCl, respectively. Stock solutions (10 μM to 100 mM) were stored at −18°C and diluted to the desired concentrations with PBS just prior to use. Ethanol did not modify twitch tension or noradrenaline-induced contractions at a concentration of 0.01% (final concentration in the medium when applying prazosin).

Results

Characterization of twitch contractions induced by electrical field stimulation

Contractions of the mouse vas deferens induced by electrical field stimulation, which averaged 539 ± 11 mg of tension per 10 mg of wet tissue ($n = 60$), were of neurogenic and sympathetic origin, as they were abolished by tetrodotoxin (100 nM) or guanethidine (5 μM, $n = 6$ for each; results not shown). Twitch contractions were also fully suppressed following desensitization of P_{2x}-purinoceptors with α,β-methylene ATP (10 μM), but were only partially inhibited (ca. 25%) by the α_1-adrenoceptor blocker, prazosin (100 nM, $n = 6$; results not shown).

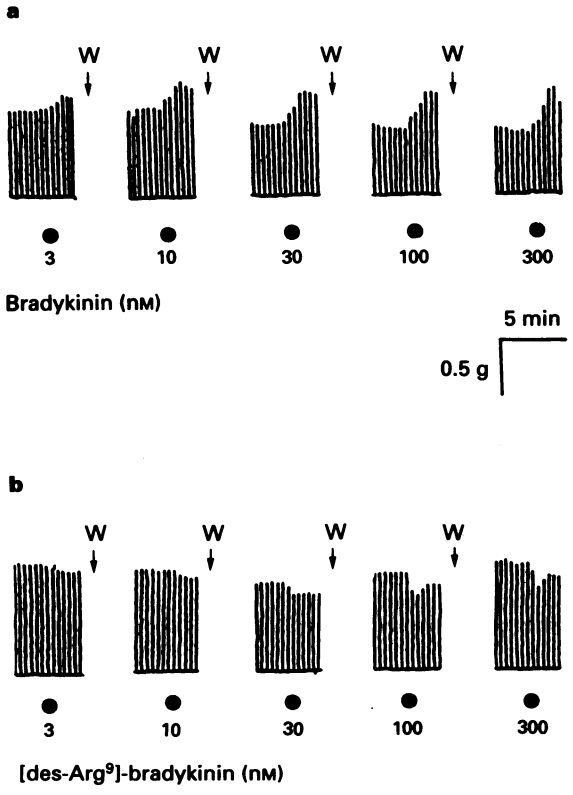

Figure 1 Typical isometric recordings showing the effect of bradykinin (a) and [des-Arg^9]-bradykinin (b) on twitch contractions induced by electrical field stimulation of the mouse isolated vas deferens. Agonists were added to the bathing medium (●) at the concentrations indicated, for 2 min at 30 min intervals. W indicates washout of the agonist. Similar recordings were obtained in another 3 to 5 experiments.

Effects of bradykinin and related peptides on neurogenic contractions

Non-cumulative addition of BK (0.1 nM to 3 μM) caused concentration-dependent potentiations of twitch contractions of the vas deferens (Figures 1 and 2). In the presence of captopril (3 μM), the EC_{50} for this effect was 4.5 nM (1.7–11.6) and the E_{max} 315 ± 19 mg per 10 mg of wet tissue (Table 1). Moreover, repeated additions of BK (30 nM), at 1 h intervals, induced reproducible increases of twitch contractions for up to 8 h after setup ($n = 3$; results not shown). In experiments carried out in the absence of captopril, the potency of BK was reduced 9 fold (EC_{50} 42.9 nM, 29.2–62.8; E_{max} 307 ± 32; $n = 6$). Potentiations of neurogenic twitch contractions were also observed in response to Lys-BK, [Hyp^3]-BK, Met,Lys-BK and the selective B_2 receptor agonist, [Tyr(Me)^8]-BK (0.1 nM to 1 μM), all of which exhibited EC_{50}s and E_{max}s similar to those of BK (Table 1).

In sharp contrast, the selective B_1 receptor agonist, [des-Arg^9]-BK (0.3 to 100 nM) caused concentration-dependent inhibitions of twitches (Figures 1 and 2). The IC_{50} for this effect of [des-Arg^9]-BK was 13.7 nM (10.4–16.1) and the I_{max} 171 ± 11 (Table 1). In other experiments, successive challenges with [des-Arg^9]-BK (300 nM) at 1 h intervals, starting 1 h after setup, induced reproducible depressions of responses to electrical field-stimulation for up to 8 h ($n = 4$; results not shown).

Effects of selective B_1 and B_2 receptor antagonists on responses to bradykinin and [des-Arg^9]-bradykinin

Prior incubation with the selective B_2 receptor antagonists, Hoe 140 (1 to 10 nM) or NPC 17731 (1 to 30 nM), caused parallel rightward displacements of the curve to BK-induced twitch potentiation, yielding apparent pA_2 values (mean ± s.e.mean) of 9.65 ± 0.09 and 9.08 ± 0.13, respectively (Figure 3). The slope values (mean and 95% confidence limits) of the Schild plot regression lines were not statistically different from unity [Hoe 140 1.1 (0.9–1.4); NPC 17731 0.9 (0.8–1.1)]. Neither antagonist displayed partial agonist activity up to 100 nM ($n = 5$; results not shown). Moreover, Hoe 140 and NPC 17731 (100 nM) did not affect twitch potentiations induced by substance P (3 nM) or endothelin-1

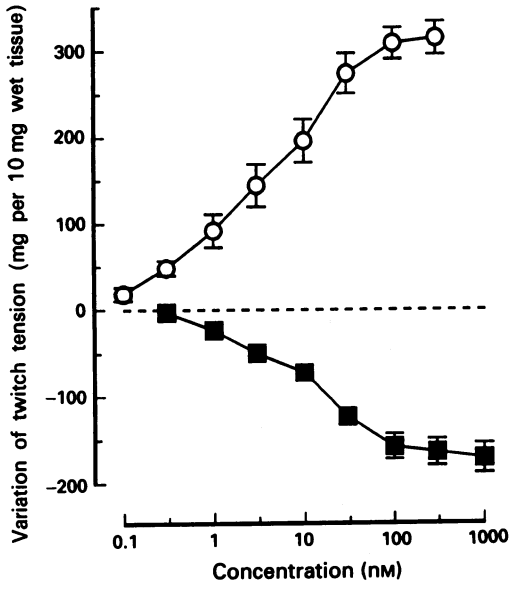

Figure 2 Mean concentration-response curves for the potentiating effect of bradykinin (○) or the inhibitory effect of [des-Arg^9]-bradykinin (■) on twitch contractions evoked by electrical field stimulation of the mouse isolated vas deferens. Values are mean ± s.e.mean of 4 to 6 experiments.

Table 1 Effects of kinins on contractions of the mouse vas deferens induced by electrical field-stimulation

Agonist	*Condition*	n	EC_{50} (nM)[a]	E_{max}[b]
BK	Alone	6	42.9 (29.2–62.8)	+307 ± 32
	+ Captopril 3 μM	6	4.5 (1.7–11.6)	+315 ± 19
Lys-BK	+ Captopril 3 μM	6	5.0 (3.3–7.4)	+332 ± 36
[Tyr(Me)^8]-BK	+ Captopril 3 μM	6	9.5 (5.7–15.7)	+367 ± 50
Met,Lys-BK	+ Captopril 3 μM	6	2.1 (1.0–3.1)	+292 ± 34
[Hyp^3]-BK	+ Captopril 3 μM	6	2.1 (1.1–3.7)	+268 ± 29
[des-Arg^9]-BK	+ Captopril 3 μM	4	13.7 (10.4–16.1)[§]	−171 ± 11[§§]

[a]Geometric means accompanied by 95% confidence limits. [b]Changes in twitch contraction amplitude, expressed in mg of tension per 10 mg wet tissue. [§] IC_{50} for twitch depression. [§§] I_{max} for twitch depression.

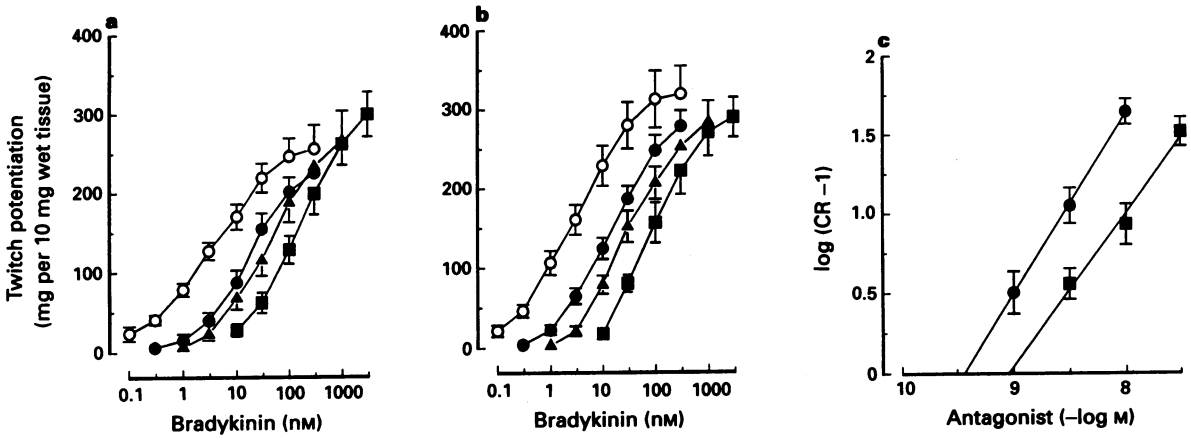

Figure 3 Influence of the selective B_2 receptor antagonists, Hoe 140 and NPC 17731, on bradykinin-induced potentiation of twitch contractions evoked by electrical field stimulation in the mouse isolated vas deferens. Mean concentration-response curves for bradykinin in the absence (○) or in the presence of (a) Hoe 140: 1 (●), 3 (▲) or 10 nM (■), or of (b) NPC 17731: 3 (●), 10 (▲) or 30 nM (■). Panel (c) shows the Schild plots for the blockade of bradykinin-induced twitch potentiation by Hoe 140 (●) or NPC 17731 (■). CR indicates the ratio of EC_{50}s obtained in the presence and absence of the antagonist. Values are mean ± s.e.mean of 6 experiments.

endothelin-1 (1 nM), but virtually abolished similar responses induced by BK (300 nM) or Lys-BK (300 nM) (Figure 4). It is important to point out, however, that neither of these B_2 receptor antagonists, at 100 nM (Figure 4) or at 1 μM ($n = 3$; results not shown), actually reversed the potentiating effect of BK into a depressor effect on twitch contractions, i.e. they did not unmask an inhibitory effect of the agonist.

As shown in Figure 5, the selective B_1 receptor antagonist [Leu^8,des-Arg^9]-BK (1 μM) significantly potentiated the effects of higher concentrations of BK (30 to 300 nM), i.e. induced further augmentation of neurogenic contractions. Furthermore, [Leu^8,des-Arg^9]-BK (1 μM) abolished the inhibition of twitch contractions of the mouse vas deferens caused by [des-Arg^9]-BK (300 nM) (Figure 6), without modifying similar twitch depressions induced by clonidine (1 nM; control 429 ± 38, plus antagonist 394 ± 48; $n = 4$) or morphine (300 nM; control 246 ± 37, plus antagonist 251 ± 26; $n = 3$). On the other hand, twitch depression induced by des-Arg^9-BK (300 nM) was not modified by Hoe 140 (30 nM) or NPC 17731 (100 nM) (Figure 6).

Effects of bradykinin and [des-Arg^9]-bradykinin on contractions induced by ATP and noradrenaline

In non-stimulated preparations, BK (100 nM) increased contractions induced by ATP (100 μM), but did not alter responses to noradrenaline (10 μM; Figure 7). The potentiation of ATP-induced contractions by BK was abolished by preincubation with the selective B_2 receptor antagonists, Hoe 140 (10 nM) or NPC 17731 (30 nM), whereas it was not affected by the selective B_1 receptor antagonist, [Leu^8,des-Arg^9]-BK (1 μM; $n = 3$ in each case; Figure 7a). In contrast to BK, [des-Arg^9]-BK did not modify contractions induced by ATP or noradrenaline (Figure 7b).

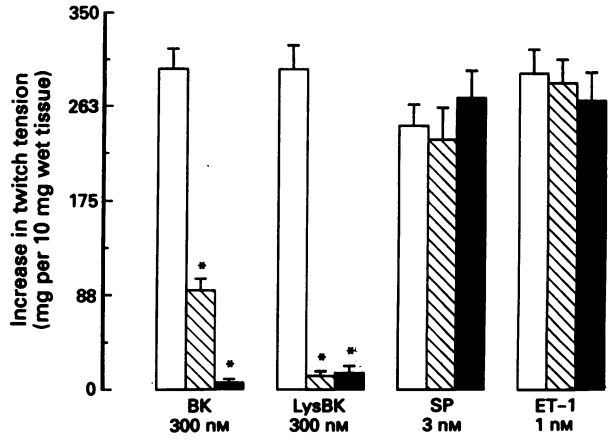

Figure 4 Influences of the selective B_2 antagonists Hoe 140 (100 nM) and NPC 17731 (100 nM) on the potentiation of contractions evoked by electrical field stimulation of the mouse vas deferens induced by bradykinin (BK, 300 nM), Lys-bradykinin (300 nM), substance P (SP, 3 nM) or endothelin-1 (ET-1, 1 nM). Values shown represent responses in the absence (open columns) or presence of Hoe 140 (hatched columns) or NPC 17731 (solid columns) and are the mean ± s.e.mean of 6 experiments. Significance of difference from control value: * $P < 0.05$ (ANOVA followed by Student's paired t test).

Discussion

The present results demonstrate that BK and related peptides modulate sympathetic neurotransmission in the mouse vas deferens in distinct ways, depending on the selectivity of the

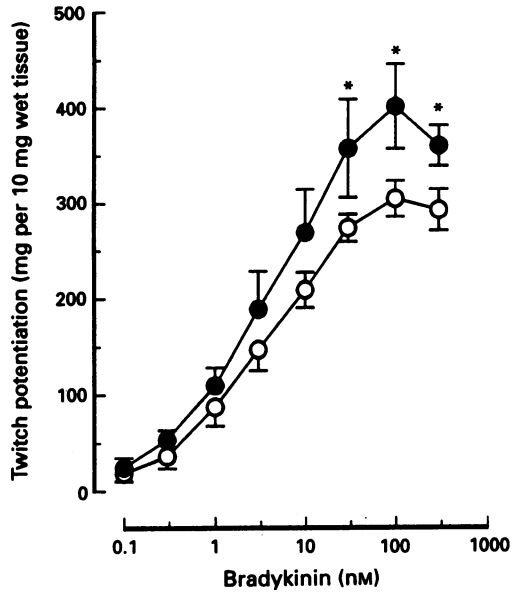

Figure 5 Influence of the selective B_1 receptor antagonist, [Leu^8,des-Arg^9]-BK, on the potentiation of contractions evoked by electrical field stimulation of the mouse vas deferens induced by bradykinin. Mean concentration-response curves for bradykinin in the absence (○) or presence (●) of [Leu^8,des-Arg^9]-BK (1 μM, added 5 min prior to each agonist addition). Values are mean ± s.e.mean of 5 experiments. Significance of difference from control value: * $P<0.05$ (ANOVA followed by Student's paired t test).

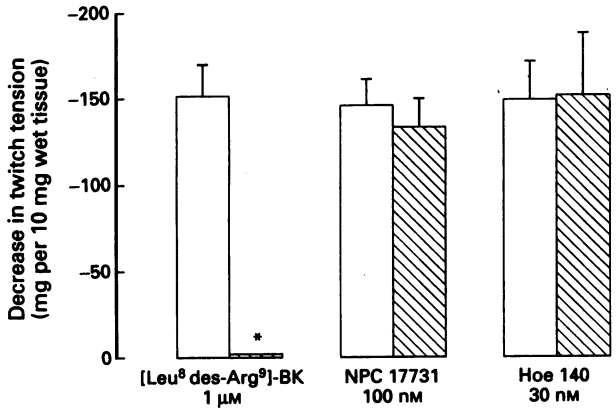

Figure 6 Influence of the selective B_1 receptor antagonist, [Leu^8,des-Arg^9]-BK and of the selective B_2 receptor antagonists, Hoe 140 and NPC 17731, on the inhibitory effect of [des-Arg^9]-bradykinin (300 nM) on twitch contractions induced by field stimulation of the mouse vas deferens. Responses to [des-Arg^9]-bradykinin were obtained in the absence (open columns) or presence (hatched columns) of an antagonist, added 5 min before at the concentration indicated. Values are mean ± s.e.mean of 5 experiments. Significance of difference from control value. * $P<0.001$ (ANOVA followed by Student's paired t test).

agonist towards B_1 or B_2 receptors. Thus, BK, Lys-BK, [Hyp^3]-BK, Met,Lys-BK and the selective B_2 receptor agonist [Tyr(Me)^8]-BK all caused pronounced potentiation of twitch responses, with potencies comparable to those found in other tissues known to express B_2 receptors (Regoli & Barabé, 1980; Bhoola *et al.*, 1992; Hall, 1992). On the other hand, the selective B_1 receptor agonist, [des-Arg^9]-BK, which is inactive in the rat vas deferens (Llona *et al.*, 1987; Asghar *et al.*, 1993), caused a graded inhibition of neurogenic responses of the mouse vas deferens. Another difference between the two species concerns the musculotropic action of BK, which is virtually non-existent in the mouse vas deferens, but robust in the rat vas deferens, particularly in the epididymal portion

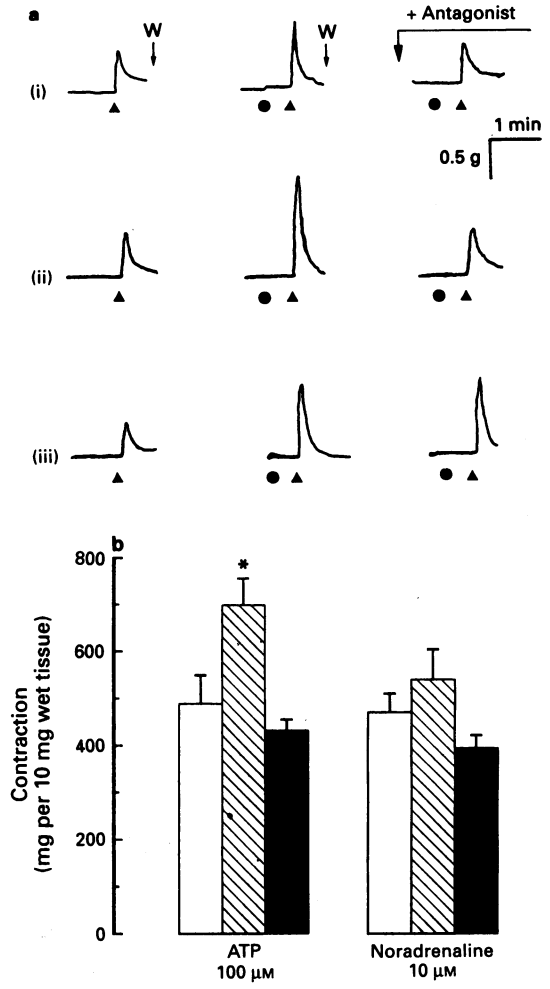

Figure 7 Effects of bradykinin and [des-Arg^9]-bradykinin on contractions induced by ATP and noradrenaline in non-stimulated mouse vas deferens. Panel (a) shows typical isometric recordings of the contractions induced by ATP (100 μM; ▲) alone, in the presence of bradykinin (100 nM added 30–45 s beforehand; ●), and in the presence of bradykinin plus Hoe 140 (10 nM; *i*), NPC 17731 (30 nM; *ii*) or [Leu^8,des-Arg^9]-bradykinin (1 μM; *iii*). Antagonists were added to the medium 5 min before bradykinin. Challenges with ATP were conducted at 30 min intervals and W indicates washout. Similar results were obtained in at least 2 additional experiments of each kind. Panel (b) shows contractions induced by ATP (100 μM) and noradrenaline (10 μM) in the absence (open columns) or in the presence of bradykinin (100 nM; hatched columns) or [des-Arg^9]-bradykinin (300 nM; solid columns). Values are mean ± s.e.mean of 4 to 7 experiments. Significance of difference from control value: * $P<0.05$ (ANOVA followed by Student's paired t test).

(Huidobro-Toro *et al.*, 1986; Rifo *et al.*, 1987; Llona *et al.*, 1987; Donoso & Huidobro-Toro, 1989; Asghar *et al.*, 1993).

The view that BK potentiates neurogenic twitches via B_2 receptors is further substantiated by results showing that Hoe 140 and NPC 17731, two highly potent and selective B_2 receptor antagonists (Hock *et al.*, 1991; Lembeck *et al.*, 1991; Kyle & Burch, 1992; 1993; Burch *et al.*, 1993; Corrêa & Calixto, 1993), induced graded rightward displacements of the twitch potentiation curve to BK without modifying the agonist's E_{max}. Furthermore, Schild regression analysis yielded straight lines with slopes not statistically different from unity, suggesting that BK and both antagonists were interacting with a homogeneous population of receptors, and that Hoe 140 and NPC 17731 act in purely competitive fashion (Kenakin, 1993). Blockade of B_2 receptors with Hoe 140 and NPC 17731 in some tissues is characterized by rightward displacement of the curve to BK allied to a

marked depression of the maximum response (Rhaleb *et al.*, 1992; Griesbacher & Lembeck, 1992; Field *et al.*, 1992; Trifilieff *et al.*, 1993). This discrepancy may be explained by the fact that neither Hoe 140 nor NPC 17731 display any partial agonist activity in the mouse vas deferens. Alternatively, it may result from the different antagonist incubation periods employed. The actions of Hoe 140 and NPC 17731 against responses to BK (and Lys-BK) were clearly specific, as, at concentrations 100 to 300 fold higher than their respective apparent K_B values for B_2 receptors, they did not affect similar twitch potentiations induced by endothelin-1 or substance P.

To our knowledge, the present study is the first to characterize successfully functional B_2 receptors in a murine tissue by use of highly selective antagonists. The apparent pA_2 value for Hoe (9.65) against BK-induced twitch potentiation in the mouse vas deferens is 10 fold larger than that found in an analogous study in the rat vas deferens (Asghar *et al.*, 1993), but is in the same general range found in other tissues expressing B_2 receptors, including guinea-pig and human ileum, rabbit jugular vein, human and hamster urinary bladder and guinea-pig taenia caeci (Hock *et al.*, 1991; Perkins *et al.*, 1991; Field *et al.*, 1992; Hall *et al.*, 1992; Rhaleb *et al.*, 1992; Griesbacher & Lembeck, 1992; Medeiros & Calixto, 1993). The same applies to the apparent pA_2 value we obtained for NPC 17731 (9.08), which is similar to that found in guinea-pig ileum (Kyle & Burch, 1992). Very recently, Hess *et al.* (1994) reported that cloned murine and human B_2 receptors, expressed in Chinese hamster ovary cells, share the same receptor-coupling mechanisms and exhibit similar affinities for B_2 receptor agonists, yet differ markedly in their affinities for B_2 receptor antagonists. The authors concluded that both species express a single B_2 receptor, despite the differences in antagonist binding which are possibly due to species differences in the gene encoding the receptor, as has been shown for other receptor types (Hall *et al.*, 1993).

The kinin receptor mediating the twitch-depressor effect of [des-Arg^9]-BK in the mouse vas deferens is most probably of the B_1 type, as it was resistant to blockade by Hoe 140 or NPC 17731, but was inhibited by the selective B_1 receptor antagonist, [Leu^8,des-Arg^9]-BK, which did not influence twitch depressions induced by clonidine or morphine. Unfortunately, it was not possible to determine the apparent pA_2 value for this effect of [Leu^8,des-Arg^9]-BK, because the relatively small maximal inhibitory response to [des-Arg^9]-BK led to considerable variability in the extent of blockade afforded by low concentrations of the antagonist. The finding that [Leu^8,des-Arg^9]-BK enhanced slightly, but significantly, the potentiation of twitch contractions induced only by high concentrations of BK suggests that the inhibitory B_1 receptors present in the mouse vas deferens display low affinity for BK. Furthermore, the contribution of such B_1 receptors to the overall effect of BK appears to be modest, as BK-induced twitch depressions could not be observed even in presence of a large concentration of the B_2 receptor antagonists, Hoe 140 or NPC 17731 (up to 1 μM).

At least part of the B_2 receptors mediating twitch potentiation appear to be located postjunctionally, as BK also potentiated the responses to an equieffective concentration of exogenous ATP, but not noradrenaline. Furthermore, this effect was also blocked by NPC 11731 and Hoe 140, but not by [Leu^8,des-Arg^9]-BK. Nevertheless, B_2 receptors may also be present on sympathetic nerve terminals of the mouse vas deferens, since BK enhances the electrically-evoked release of [^{3}H]-noradrenaline in this preparation (Llona *et al.*, 1991). Conversely, the location of the B_1 receptors mediating twitch inhibition seems to be mainly prejunctional as [des-Arg^9]-BK failed to affect contractions induced by either ATP or noradrenaline.

Although B_1 receptors in most tissues appear to be inducible (for review see Marceau & Regoli, 1991; Hall, 1992), a variable but significant proportion of constitutive B_1 receptors has been proposed to exist in the rat duodenum (Boschcov *et al.*, 1984), stomach fundus (Calixto & Medeiros, 1992) and portal vein (Campos & Calixto, 1994). It is tempting to propose that both B_1 and B_2 receptors in the mouse vas deferens are constitutive, as responses mediated by these receptor types were obtained immediately after the end of the equilibration period and remained reproducible for up to 8 h after setup. However, the present evidence is still insufficient to make such a proposal.

In conclusion, we have shown that, in contrast to what has been reported in rat vas deferens, kinins exert both facilitatory and inhibitory influences on sympathetic neurotransmission in the mouse vas deferens. While the facilitatory influence seems to involve both pre- and postjunctional B_2 receptors, twitch inhibition is mediated by stimulation of B_1 receptors, which are presumably prejunctional. Further studies are in progress to clarify the cellular mechanisms triggered by activation of both types of kinin receptors and their possible relevance for transmission at this autonomic neuromuscular junction.

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