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Characterisation of bradykinin B_1 and B_2 receptors using rat isolated vas deferens

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Abstract

The actions of bradykinin and its metabolite des-Arg[9] bradykinin are mediated through activation of bradykinin B_2 and B_1 receptors, respectively. The aim of the present study was to characterize native bradykinin receptors focusing on induction and desensitization using rat isolated vas deferens. Tissues were mounted in organ baths for isometric recordings and neurogenically mediated contractions were evoked by electrical stimulation. Des-Arg[9] bradykinin enhanced the magnitude of the electrically evoked contractions and this effect (which was sensitive to blockade by the peptide bradykinin B_1 receptor selective antagonist B9858, Lys-Lys-(Hyp[3],Cpg[5],D-Tic[7],Cpg[8])des-Arg[9] bradykinin) was only observed following a pre-incubation period and was greatest following 5 h of pre-incubation. Bradykinin also potentiated neurogenically evoked contractions and this effect was sensitive to blockade by Hoe 140 (D-Arg(Hyp[3],Thi[5],D-Tic[7],Oic[8])bradykinin, a peptide bradykinin B_2 receptor antagonist) and was present without pre-incubation but was increased by pre-incubation and reached maximum at the 5-h incubation time point. Responses to bradykinin were larger than those to des-Arg[9] bradykinin. Bradykinin responses did not show desensitization on repeated agonist stimulation. These data confirm in rat isolated vas deferens bradykinin B_2, but not B_1, receptors are constitutively expressed, that both receptor populations are inducible and B_2 receptors do not exhibit desensitization.

Keywords: Bradykinin receptor; Induction; Desensitisation; (Rat); Vas deferens

1. Introduction

In rodents, bradykinin is generated via activation of the kallikrein–kinin system, followed by subsequent metabolism of bradykinin by cyclopeptidases generating the pharmacologically active metabolite des-Arg[9] bradykinin. Responses to bradykinin and des-Arg[9] bradykinin are mediated through bradykinin B_2 and B_1 receptors, respectively (Regoli et al., 1997). In most species, bradykinin B_2 receptors are constitutively expressed in normal tissues and have a wide distribution (for reviews, see Hall and Morton, 1997; Rupniak et al., 2000). Bradykinin B_2 receptors serve a variety of functions and particularly those associated with pain and inflammation resulting from tissue damage, microbial infection and tissue lesions. Bradykinin B_1 receptors (although less well characterised) have also been detected at similar sites and overall seem to mediate similar effects (including pain and inflammation). However, a notable difference is that until recently B_1 receptors (unlike B_2 receptors) were regarded as not being constitutively expressed in normal tissues from most species (except dog), but were inducible following tissue injury/damage/inflammation (Marceau, 1995; Regoli et al., 1997; Rupniak et al., 2000; Bock and Longmore, 2000).

Although it has been implied that both these receptor subtypes are involved in pain and inflammatory processes, it has been suggested that bradykinin B_2 receptors play an acute, early stage role, whereas bradykinin B_1 receptors play a chronic, maintenance role since induction is necessary by the tissue injury/damage process itself (Perkins et al.,1993; Manning et al., 1991; Cruwys et al., 1994; Perkins and Kelly, 1993; Correa and Calixto, 1993; Marceau, 1995). However, it has been demonstrated that B_2 receptors can be upregulated in cultured isolated rat dorsal root ganglion neurones (Eckert et al., 1999) and, on the other hand, constitutive expression of B_1 receptors (either as mRNA/protein or functional response)

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has been demonstrated in the rodent sensory nervous system (Wotherspoon and Winter, 2000; Pesquero et al., 2000; Ma et al., 2000) and human brain and spinal cord (Raidoo and Bhoola, 1997; Mahabeer et al., 2000).

The aim of the present study was to characterise the pharmacology of bradykinin B_1 and B_2 receptors using neurogenic responses in electrically stimulated isolated rat vas deferens and to examine the induction and desensitisation of these receptors. This tissue was chosen because in rodent it has been previously shown to express both bradykinin receptor subtypes (Maas et al., 1995).

2. Material and methods

2.1. Isometric tension recordings

Isolated vas deferens were obtained from male Wistar rats (200–300 g) following euthanasia. Tissues were cleared of surrounding connective and adipose tissue and mounted for isometric tension recording (PowerLab ADI) in an organ bath containing physiological saline solution, aerated with carbogen (95% O_2/5% CO_2; pH 7.4, 37 °C). A resting tension of 1 g was applied, and the tissue was allowed to equilibrate for 30 min prior to transmural electrical field stimulation. Two platinum electrodes were inserted at the top and bottom of the organ bath and connected to an electrical stimulator (MultiStim System-D330, Digitimer). Trains of electrical pulses were delivered at 20 Hz, 0.5 ms (pulse width), 1-s duration at 40-s intervals using threshold voltage (10–15 V). Once a contractile twitch response was generated by electrical stimulation, the tissue was given a further 30 min for the twitch response to stabilise. In a series of preliminary experiments to establish whether the contractions were sympathetically mediated, we examined the inhibitory effects of the α_1 and α_2 adrenoceptor ligands prazosin and clonidine. For the main experiments, prior to peptide additions the tissues were incubated (10 min) with captopril (10 μM) and thiorphan (10 μM) to inhibit angiotensin-converting enzyme and neutral endopeptidase, respectively, and prevent peptide metabolism. Agonist peptides (either des-Arg^9 bradykinin or bradykinin) were added to the tissue baths in a cumulative manner (1 nM–3 μM). For des-Arg^9 bradykinin, it was necessary to allow a 5-h pre-incubation period (see below) to induce a response, since in naïve or nonincubated tissues there was no-response to des-Arg^9 bradykinin. For antagonist studies, the selective bradykinin B_1 and B_2 receptor peptide antagonists B9858 (Lys-Lys-(Hyp^3,Cpg^5,D-Tic^7,Cpg^8)des-Arg^9 bradykinin, 1, 10 or 100 nM) or Hoe 140 (D-Arg(Hyp^3,Thi^5,D-Tic^7,Oic^8)-bradykinin, 10 nM) or clonidine (α_2 adrenoceptor agonist, 300 nM) were added 20 min prior to the kinin agonists using a matched pairs protocol, where one vas deferens was treated with vehicle and the other from the same animal was treated with the antagonist. For desensitisation experiments, an initial concentration effect curve to bradykinin was obtained (control $t = 0$ h), the tissues were washed until baseline tension was re-established, followed by a 20-min re-equilibration period and then the concentration effect curve was repeated twice (such that each curve was separated by approximately 30 min). It was not possible to perform similar experiments with des-Arg^9 bradykinin since there was no response to the agonist at the 0-h time point.

For the receptor induction experiments, tissues were pre-incubated (0, 2, 5 or 24 h) in 10 ml oxygenated physiological saline at 37 °C and were then mounted in the organ baths for isometric tension recording as described above.

2.2. Analysis of data

The agonist-evoked potentiation of the electrically driven contractile twitch was expressed as the percentage increase in the twitch response above the basal twitch height (see Fig. 1). Concentration–effect curves for the mean responses for des-Arg^9 bradykinin and bradykinin were fitted to a model using weighted least squares nonlinear regression analysis and the equation:

$$E = E_{max}/(1 + (EC_{50}/\text{agonist concentration})^{nH})$$

where E_{max} is the maximum contraction evoked by each agonist (relative to height of the basal twitch contraction),

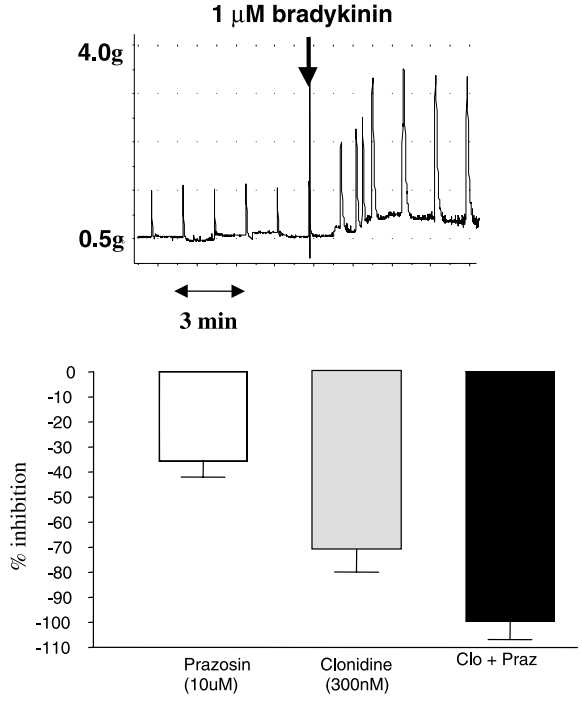

Fig. 1. Upper panel: Representative recording of rat isolated vas deferens showing tension (g) against time (min) during application of electrical field stimulation (20 Hz, 0.5 ms, 1 s duration at 40 s intervals, voltage ranging between 10 and 15 V) to evoke 'twitch' contractile responses which were potentiated by bradykinin peptides. Lower panel: antagonism of the twitch responses by application of clonidine (300 nM) or prazosin (10 μM) and simultaneous application of these compounds. Dates are mean values ($n = 4$), ± S.E.M.

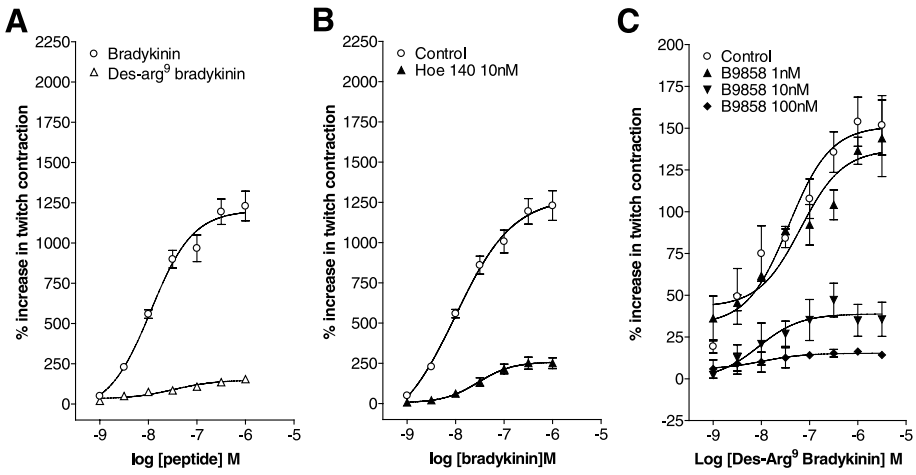

Fig. 2. Left panel: Comparison of the magnitude of contractions evoked by bradykinin (○) and des-Arg^9 bradykinin (△) in the presence of captopril and thiorphan (tissue incubated for 5 h prior to des-Arg^9 bradykinin concentration response curve). Middle panel: bradykinin concentration effect curves following incubation with vehicle (○) or Hoe 140 (10 nM, ▲) in the presence of captopril and thiorphan and responses to des-Arg^9 bradykinin following pre-incubation with vehicle (○) or B9858 (1(▲), 10 (▼), 100 (♦) nM) in the presence of captopril and thiorphan (tissues were pre-incubated for 5 h prior to experiment). Points represent mean values ($n=4$), error bars indicate ± S.E.M. Curves were fitted using nonlinear regression analysis.

EC_{50} (with 95% confidence limits) is the half maximally effective concentration and nH is the Hill coefficient (GraphPad Prism 2.0b).

2.3. Drugs and solutions

The physiological salt solution had the following composition: NaCl 118 mM, KCl 4.7 mM, KH_2PO_4 1.19 mM, $NaHCO_3$ 23.5 mM, $MgSO_4$ 1.19 mM, glucose 11.1 mM, $CaCl_2$ 2.5 mM. Bradykinin and des-Arg^9 bradykinin peptides were obtained from Bachem (UK); captopril, clonidine, Hoe 140 (D-Arg(Hyp^3,Thi^5,D-Tic^7,Oic^8)bradykinin), prazosin, and thiorphan were from Sigma. B9858 (Lys-Lys-(Hyp^3,Cpg^5,D-Tic^7,Cpg^8)des-Arg^9 bradykinin) was synthesised by Medicinal Chemistry (Merck Research Laboratories, USA).

3. Results

A representative trace is shown in Fig. 1A. The twitch contraction was inhibited by clonidine or prazosin. Preliminary concentration effect curves (data not shown) showed that clonidine 300 nM and prazosin (10 μM) represented approximately EC_{95-100} and therefore these concentrations were used in subsequent experiments. When added simultaneously clonidine (300 nM) and prazosin (10 μM) completely abolished the neurogenic contractions (see Fig. 1B). Cumulative addition of des-Arg^9 bradykinin and bradykinin (using 5 h pre-incubation and non-pre-incubated tissues, respectively) produced an increase in the magnitude of the contractions evoked by each train of electrical stimuli (neurogenic response) and produced a smaller increase in the underlying baseline tension (musculotropic response). A representative effect of bradykinin is shown in Fig. 1A. All results refer to the effects of kinins on the neurogenic response. Mean concentration effect curves are shown in Fig. 2 and the EC_{50} and E_{max} values are summarised in

Table 1
Comparison of the EC_{50} (nM with 95% confidence limits) and E_{max} values (with asymptotic error) derived from the curve fitting procedure for bradykinin and des-Arg^9 bradykinin in the absence and presence of Hoe 140, B9858 or clonidine (300 nM)

Agonist/antagonist	EC_{50} with 95% C.L. (nM)	E_{max} ± S.E.M. (%)
Bradykinin	12 (7–19)	1214 ± 40
Bradykinin + Hoe 140 (10 nM)	28 (12–66)	267 ± 20
Des-Arg^9 bradykinin	37 (17–99)	151 ± 9
Des-Arg^9 bradykinin + B9858 (10 nM)	9 (1–91)	39 ± 5
Bradykinin + clonidine	77 (5–1200)	143 ± 10
Des-Arg^9 bradykinin + clonidine	95 (6–1500)	14 ± 1

Table 2
Comparison of the EC_{50} (nM with 95% confidence limits) and E_{max} values (with asymptotic error) derived from the curve fitting procedure for bradykinin and des-Arg^9 bradykinin with increasing periods of receptor induction (0, 2, 5, 24 h)

Agonist/antagonist	EC_{50} with 95% C.L. (nM)	E_{max} ± S.E.M. (%)
Bradykinin control (0 h)	11 (8–15)	1081 ± 25
Bradykinin (2 h)	17 (8–36)	1371 ± 59
Bradykinin (5 h)	13 (6–31)	1996 ± 106
Bradykinin (24 h)	38 (8–175)	1238 ± 108
Des-Arg^9 bradykinin (0 h)	11 (1–139)	10 ± 3
Des-Arg^9 bradykinin (2 h)	1300 (28–62,000)	34 ± 26
Des-Arg^9 bradykinin (5 h)	37 (14–99)	151 ± 9
Des-Arg^9 bradykinin (24 h)	167 (10–2700)	46 ± 11

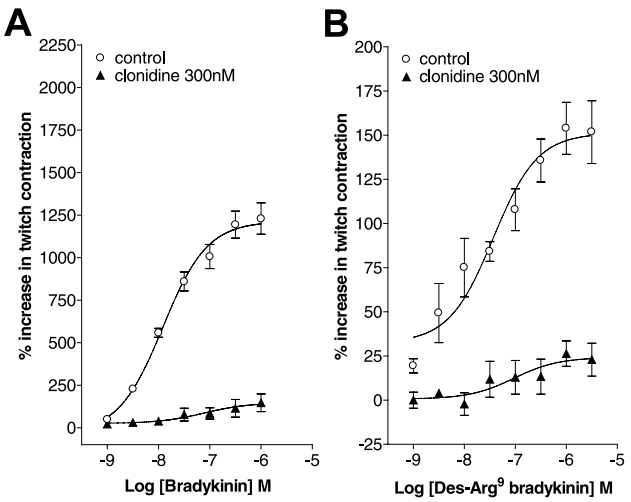

Fig. 3. Effect of clonidine (300 nM) effect on responses to bradykinin (panel A) and des-Arg^9 bradykinin (panel B). Vehicle-treated tissues (○) and clonidine-treated tissues (▲). Points represent mean values ($n=4$), error bars indicate ± S.E.M. Curves were fitted using nonlinear regression analysis.

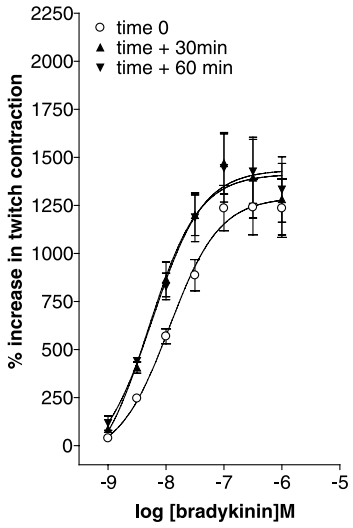

Fig. 4. Effect of repeated bradykinin concentration-effect curves, first curve control $t=0$ min (○), second curve $t=+30$ min (▲) and third curve $t=+60$ min (▼). Points represent mean values ($n=4$), error bars indicate ± S.E.M. Curves were fitted using nonlinear regression analysis.

Table 1. Comparison of the magnitude of the responses to the peptide agonists showed that the E_{max} response for des-Arg^9 bradykinin was significantly (approximately 9-fold) lower than the E_{max} for bradykinin (see Fig. 2 and Table 1). The responses to des-Arg^9 bradykinin and bradykinin were inhibited by B9858 (10 or 100 nM) or Hoe 140 (10 nM), respectively, both antagonists acting in a noncompetitive manner with a significant reduction in the maximal (E_{max}) response, while mean EC_{50} values remained statistically unchanged from the control values (see Fig. 2 and Table 1).

Pre-incubation of tissues caused a time-dependent increase in the magnitude of agonist response (see Table 2). For des-Arg^9 bradykinin, the response at time 0 h was small ($E_{max}=10\pm3$), peaked at 5 h ($E_{max}=151\pm9$ and where the potency of des-Arg^9 bradykinin was 37 nM in line with the reported potency at rodent bradykinin B_1 receptors; Regoli et al., 1998) and fell at 24 h ($E_{max}=46\pm11$). For bradykinin there was a substantial effect on the twitch response without pre-incubation ($t=0$ h, $E_{max}=1081\pm25$), 25), which was increased by pre-incubation (with an optimal twofold increase in E_{max} measured at the 5-h incubation time point). The EC_{50} for bradykinin was similar at all time points tested (i.e. ranged from 11 to 38 nM) and was in line with the reported potency measured at rodent bradykinin B_2 receptors (Reissman et al., 1996). The response to bradykinin following 5 h of incubation was antagonized in a noncompetitive manner by Hoe 140 (10 nM), which reduced the E_{max} from 1996 ± 106% to 336 + 58% ($n=4$).

Clonidine (300 nM) greatly reduced the E_{max} for bradykinin B_1 and B_2 receptor responses (see Fig. 3 and Table 3) but did not affect the EC_{50} values for bradykinin and des-Arg^9 bradykinin, which fell within 95% confidence limits of control values.

Repetition of the bradykinin cumulative concentration effect curve was performed at 30-min intervals (see Fig. 4). EC_{50} values did not differ significantly across the three curves. The maximal response (E_{max}) at time points 0, +30 min, and +60 min showed little variation, with a small increase over time (see Table 3) consisting with the induction of bradykinin responsiveness towards the 2-h time point.

Table 3
Effect of repeated exposure to bradykinin on EC_{50} and E_{max} values

Agonist/antagonist	EC_{50} with 95% C.L. (nM)	E_{max} ± S.E.M. (%)
Bradykinin (neurogenic) (time, 0 min)	11 (5–23)	1292 ± 63
Bradykinin (neurogenic) (time, +30 min)	5 (2–14)	1414 ± 77
Bradykinin (neurogenic) (time, +60 min)	6 (2–15)	1436 ± 73

Bradykinin concentration curves were repeated three times at 30-min intervals ($n=4$).

4. Discussion

In the present study, we used the potentiation of electrically evoked contractions in rat isolated vas deferens to characterise bradykinin B_1 and B_2 receptor pharmacology, particularly with respect to receptor induction and desensitization. In agreement with previous reports (Rifo et al., 1987; Tousignant et al., 1987; Asghar et al., 2000), bradykinin (and in the present study des-Arg^9 bradykinin) caused an increase in the underlying baseline tension and an increase in the height of electrically evoked contractions. These previous studies have characterised the changes in

baseline tension as being musculotropic in origin (i.e. direct effect on smooth muscle indicating the post-junctional localisation of bradykinin receptors) and the increase in twitch height as being neurogenic in origin (mediated via sympathetic nerve stimulation) and consistent with the expression of pre-junctional bradykinin receptors located on sympathetic nerve fibres. Whilst receptor localisation and functional studies also suggest bradykinin B_2 receptor expression on sympathetic neurones, the similar expression of functional bradykinin B_1 receptors is less well defined (see below). Therefore in the present experiments we focused on the neurogenic component of the kinin response.

Both des-Arg^9 bradykinin and bradykinin were potent agonists and potentiated the electrically evoked contractions. The observation of bradykinin-mediated increases in electrically evoked responses concurred with previously reported data in mouse and rat isolated vas deferens (Maas et al., 1995, Asghar et al., 2000), suggesting consistency of the response to the activation of B_2-receptors across these species. However, the present results differ from the study of Maas et al. (1995) where des-Arg^{9}bradykinin attenuated the twitch contractions. In the present study, responses to des-Arg^9 bradykinin and bradykinin were inhibited by B9858 and Hoe 140 (selective bradykinin B_1 and B_2 receptor antagonists, respectively) confirming that these responses were B_1 and B_2 receptor mediated. B9858 and Hoe140 are synthetic bradykinin derivatives and are good pharmacological tools. B9858 is reported in the literature to have reasonable selectivity (>30-fold, MacNeil et al., 1997) for rodent bradykinin B_1 receptors over B_2 receptors, but resistant to peptidase degradation and have little if no residual agonist activity (Regoli et al., 1998). Similarly, Hoe 140 is resistant to peptidase degradation and has good, selective affinity for rodent bradykinin B_2 receptors (Regoli et al., 1998). In the present study, Hoe 140 alone did not cause any alteration in tension indicating little if no partial agonist activity. Therefore the present findings confirm those of previous studies which were conducted several years ago before the availability of these good pharmacological tools (Rifo et al., 1987; Tousignant et al., 1987). In the present study, B9858 and Hoe140 acted as noncompetitive antagonists producing a significant insurmountable, reduction of E_{max}. Asghar et al. (2000) reported different antagonistic effects, with Hoe140 acting as a competitive and noncompetitive antagonist on the musculotropic and neurogenic components, respectively. These authors suggested this was due to the existence of different activation states of the bradykinin B_2 receptor. It is possible that differences in the type of Hoe 140 antagonism seen in the present study may reflect different activation states induced by small differences in experimental protocols.

The neurogenic contraction in rat isolated vas deferens has been previously well characterised and generally accepted as being mediated through sympathetic nerve stimulation and can be inhibited by α_1 adrenoceptor antagonists such as prazosin (acting post-junctionally) and by α_2 adrenoceptor agonists such as clonidine (acting at inhibitory pre-junctional receptors; Rifo et al., 1987; Tousignant et al., 1987). A variety of electrical stimulation paradigms have been used to drive sympathetic transmission, ranging from continuous low-frequency stimulation (e.g. 0.1 Hz; e.g. see Asghar et al., 2000; Knight et al., 2001) to frequencies as high as 16 or 30 Hz delivered intermittently (Hughes, 1973; Greenberg et al., 1991). The parameters used in the present study delivered stimuli at 20 Hz producing neurogenic contractions consistent over time and which were able to be potentiated by kinin agonists. Similarly to previous studies, the sympathetic nature was confirmed using clonidine (300 nM EC_{95-100}) consistent with the concentrations used in noradrenaline release studies (500 nM; Knight et al., 2001) and suggested to act by blocking calcium currents in sympathetic nerve fibres (for references, see Knight et al., 2001).

The ability of clonidine to inhibit the potentiation of the contractions mediated through bradykinin B_1 and B_2 receptors suggests that these responses were mediated through pre-junctional bradykinin receptors which regulate noradrenaline release. From the literature, bradykinin B_2 receptors are present on peripheral terminals of sympathetic and sensory nerve fibres and B_1 receptors are present on primary sensory neurones (Rupniak et al., 2000; Ma et al., 2000; Pesquero et al., 2000; Wotherspoon and Winter, 2000), although little conclusive evidence exists for the activation of sympathetic/sensory nerve fibres by bradykinin B_1 receptor agonists. However, in the present studies using rat isolated vas deferens, the potentiating actions of des-Arg^9 braydykinin and bradykinin on neurogenic contractions, mediated solely through pre-junctional receptors, are difficult to reconcile since the ability of des-Arg^9 bradykinin (and to a lesser extent bradykinin) to potentiate neurogenic responses was greater following pre-incubation (suggesting receptor induction). Generally, induction of bradykinin B_1-receptor-mediated responses relies on de novo protein synthesis (see Rupniak et al., 2000) and this is unlikely to occur in the present experiments since the induction was performed post-isolation (and the sympathetic nerve terminals were severed from the cell bodies where mRNA translation and protein synthesis take place). It is possible that the induction process may recruit previously formed nonfunctional kinin receptor protein and indeed there are a few literature reports where bradykinin B_1 receptor induction is not abolished by inhibition of protein synthesis (for review, see Bock and Longmore, 2000). Alternatively, the potentiating effects of the peptide agonists could be mediated post-junctionally through bradykinin receptors, which operate in synergistic manner with the actions of noradrenaline at post-synaptic α_1-adrenoceptors. Clearly, post-junctional bradykinin receptors exist as evidenced by the musculotropic response (Rifo et al., 1987; Tousignant et al., 1987). In this case, clonidine would inhibit the release of noradrenaline, minimising the synergism. Further experiments are needed for clarification.

As previously mentioned, the majority of bradykinin B_1 receptor expression is induced by conditions associated with inflammatory processes, the receptor not generally demonstrating constitutive expression and the present study showed this to be the case for bradykinin B_1-receptor-mediated responses in rat isolated vas deferens. Optimal induction of bradykinin B_1-receptor-mediated response was conferred by a 5-h incubation period, with a 24-h incubation showing a significantly lower tissue response, suggesting this length of induction adversely affected the tissue. The magnitude of the B_1 receptor response (even at the optimal induction time) was substantially lower (approximately 7–13-fold) than the B_2-receptor-mediated responses. These results imply that where the two receptors co-exist, B_2 responses will predominate over B_1 responses.

Expression of the bradykinin B_2 receptor is accepted to occur constitutively in most tissues (Rupniak et al., 2000), yet recent investigations in cultured neuronal tissue have suggested that the expression of B_2 receptors may be up-regulated under inflammatory conditions (Schmidlin et al., 1998; Eckert et al., 1999). The incubation parameters used for bradykinin B_1 receptor induction were effective in inducing B_2-receptor-mediated responses with the magnitude of the response doubling at the 5-h time point. Hoe 140 (10 nM) noncompetitively antagonised the induced response to bradykinin, confirming that the observed increase in the tissue response following incubation was B_2 receptor mediated.

The present study also showed that native bradykinin B_2 receptors were resistant to desensitisation following repeated application of bradykinin. The absence of receptor desensitisation suggested B_2 receptors may play a more prominent role in the mediation of chronic processes than is currently understood, as if they do not desensitise the receptors, and should continue to mediate responses under chronic conditions. Investigation in further tissues and species is required to determine whether the absence of desensitisation to repeated bradykinin stimulus shown in this study is representative of the general response of B_2 receptors, or simply a species/tissue specific phenomena.

To conclude, the main points raised by this investigation are as follows: (1) inducible expression of the bradykinin B_1 receptor response is observed in the rat isolated vas deferens, though the level B_1 receptor responsiveness was substantially lower than B_2 receptor responsiveness; (2) bradykinin B_2 receptor responses can be induced (shown by the doubling of E_{max} at the 5-h time point) using procedures that also upregulate B_1-receptor-mediated responses; (3) B_2 receptors are resistant to desensitisation. Collectively, these observations suggest the B_2 receptor may represent a good drug target.

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