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Hoe 140 and pseudo-irreversible antagonism in the rat vas deferens in vitro

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Abstract

The effects of bradykinin and the bradykinin B_2 receptor antagonists D-Arg-[Hyp[3],Thi[5,8],D-Phe[7]]-bradykinin (NPC 349) and D-Arg-[Hyp[3],Thi[5],D-Tic[7],Oic[8]]-bradykinin (Hoe 140) were examined in the electrically-stimulated rat vas deferens. Cumulative additions of bradykinin (1–3000 nM) produced two distinct responses: an enhancement in the magnitude of the basal electrically-induced twitch response (neurogenic response) and an increase in the baseline tension (musculotropic response). NPC 349 (10–100 μM) produced concentration-dependent surmountable rightward shifts of both the bradykinin neurogenic and musculotropic response curves. In contrast, while Hoe 140 (10–100 nM) caused an apparently surmountable antagonism of the bradykinin neurogenic response, it caused an apparent insurmountable antagonism of the bradykinin musculotropic response. Interestingly, co-incubation of Hoe 140 (30 nM) with NPC 349 (30 and 100 μM) resulted in a concentration-related upwards displacement of the Hoe 140-suppressed bradykinin musculotropic response curve. Thus, Hoe 140 can be described as a pseudo-irreversible antagonist against the bradykinin musculotropic response. No time-dependent changes were observed in the maximum bradykinin musculotropic response attainable when NPC 349 (100 μM) additions were made for the final 2 or 18 min of the Hoe 140 incubation (20 min). These findings indicate that slow reversibility of Hoe 140 from the bradykinin B_2 receptor is unlikely to be the mechanism responsible for the pseudo-irreversible antagonism of the bradykinin-induced musculotropic response. Instead, we propose an alternative explanation involving a third, unstable and inactive form of the bradykinin B_2 receptor.

Keywords: Vas deferens, rat; Bradykinin; Bradykinin receptor; Hoe 140; Antagonism, insurmountable

1. Introduction

The nonapeptide bradykinin has been studied in various tissue preparations both in vivo and in vitro where it causes a wide range of effects, including contraction and/or relaxation of smooth muscle and modification of autonomic neuromuscular transmission (Erdos, 1979; Taylor et al., 1989; Bhoola et al., 1992; Farmer and Burch, 1992; Hall, 1992). The responses to bradykinin are mediated by bradykinin receptors which have been classified into bradykinin B_1 and B_2 subtypes (Regoli and Barabé, 1980; Regoli et al., 1990, 1998). The vast majority of the physiological and pharmacological effects of bradykinin appear to be mediated by bradykinin B_2 receptors (Bathon and Proud, 1991; Bhoola et al., 1992; Hall, 1992).

In the urogenital tract, bradykinin can affect sympathetic nervous function. For example, application of bradykinin to the electrically-stimulated rat vas deferens enhances the magnitude of the electrically-driven twitches, termed the prejunctional or neurogenic response, and increases the basal tension of the muscle, called the postjunctional or musculotropic response (Huidobro-Toro et al., 1986; Llona et al., 1987; Rifo et al., 1987). These neuro-

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genic and musculotropic responses to bradykinin in the field-stimulated rat vas deferens are antagonised by the bradykinin B_2 receptor antagonist D-Arg-[Hyp^3,Thi^{5,8},D-Phe^7]-bradykinin (NPC 349) in a competitive (surmountable) manner (Rifo et al., 1987). NPC 349 and other 'first generation' antagonists for the bradykinin B_2 receptor were shown to be of weak affinity and potency (pK_B 6–7, Burch et al., 1990). However, newer bradykinin B_2 receptor antagonists show potencies up to two or three orders of magnitude greater than the earlier compounds (Hock et al., 1991; Kyle et al., 1991; Lembeck et al., 1991; Burch and Kyle, 1992; Rhaleb et al., 1992; Correa and Calixto, 1993). One such potent bradykinin B_2 receptor antagonist is D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8]-bradykinin (Hoe 140).

Although the profile of antagonism displayed by Hoe 140 is highly dependent upon the species and tissue used for assay, most studies have shown that it is predominantly an insurmountable antagonist. Thus, Hoe 140 is an insurmountable antagonist against responses to bradykinin in guinea pig ileum (Griesbacher and Lembeck, 1992), rabbit jugular vein (Rhaleb et al., 1992; Félétou et al., 1994; Marceau et al., 1994), and guinea pig trachea (Field et al., 1992; Trifilieff et al., 1993). By contrast, Hoe 140 causes surmountable antagonism in the human umbilical vein (Marceau et al., 1994). Hoe 140 has also been documented to have partial agonist activity at the sheep bradykinin B_2 receptor (Félétou et al., 1994), and in rat myometrial cells, it can behave as an inverse agonist at bradykinin B_2 receptors (Leeb-Lundberg et al., 1994). Interestingly, although Hoe 140 behaved as an inverse agonist at the human bradykinin B_2 receptor expressed in COS-7 cells, the drug was a full agonist at some mutant human forms of the bradykinin B_2 receptor (Marie et al., 1999).

In previous experiments using the in vitro electrically-stimulated rat vas deferens preparation, we observed that while Hoe 140 was a potent antagonist of bradykinin-induced effects, this antagonism was insurmountable against musculotropic responses to bradykinin, but surmountable against neurogenic responses (Asghar et al., 1993). It has been suggested that Hoe 140 may be a non-equilibrium antagonist which dissociates slowly from the bradykinin B_2 receptor to prevent full occupation of the receptors by bradykinin (Rhaleb et al., 1992; Regoli et al., 1998). In the present study, we have explored further the nature of Hoe 140-induced blockade of bradykinin receptors in the electrically-stimulated rat vas deferens.

2. Methods

2.1. Preparation of isolated vas deferens

Adult male Wistar rats (200–350 g) were killed by cervical dislocation and the vasa deferentia immediately removed. The surrounding connective and adipose tissue, and the adjoining blood vessels were removed from the vas deferens. Only the prostatic segment (1–1.5 cm) of the rat vas deferens was used in the experiments.

Each segment was immersed in a 5 ml organ bath containing Krebs solution of the following composition (mM): NaCl 118, KCL 5.4, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_2$ 25, glucose 11.1, and $CaCl_2$ 2.5. The Krebs solution was gassed with 95% O_2 and 5% CO_2 and maintained at 35°C.

For transmural electrical stimulation each segment was suspended between two parallel platinum electrodes. Electrical pulses were delivered by a multi-stimulator (Digitimer System-D330) at a frequency of one pulse every 3 s of 1 ms pulse duration and at supramaximal voltage. To record isometric muscular contractions, the segments were connected to a force transducer (Dynamometer UF1) coupled to a chart recorder (Lectromed MT8-PX).

Prior to electrical field-stimulation or the application of drugs, the vas deferens was allowed to equilibrate for 60 min with 0.5 g of basal tension. During this equilibration period, the tissue was washed with Krebs solution every 15 min and the tension re-adjusted to 0.5 g.

2.2. Bradykinin cumulative concentration–response curves

Bradykinin was added in a cumulative manner; preliminary experiments (data not shown) demonstrated that cumulative log concentration–response curves were similar to the respective curves obtained by single additions for both neurogenic and musculotropic effects of bradykinin. The method of cumulative additions of bradykinin has advantages over the single concentrations log concentration–response curves used by other investigators in the vas deferens (Huidobro-Toro et al., 1986; Rifo et al., 1987; Tousignant et al., 1987) in terms of the speed of construction of the curves (< 5 min). Furthermore, by using cumulative additions of bradykinin, it was possible to obtain three further cumulative concentration–response curves without significant change in curve location ($P > 0.05$, comparison of EC_{50} values using paired *t*-test). This was advantageous because it was feasible to add three increasing concentrations of antagonist on a single tissue, thus facilitating with tissue determinations of antagonist potency.

With regard to bradykinin B_1 receptors, we have previously shown that they do not mediate a musculotropic response nor do they influence electrically-evoked twitches in the rat vas deferens (Asghar et al., 1993).

2.3. Bradykinin receptor antagonist protocols

To evaluate the potency of the bradykinin B_2 receptor antagonists, NPC 349 and Hoe 140, cumulative log concentration–response curves to bradykinin were constructed

in the absence and presence of the test antagonist. NPC 349 (10, 30 and 100 μM) or Hoe 140 (10, 30 and 100 nM) were added to the organ bath in the absence of electrical stimulation, 20 min prior to re-construction of the bradykinin cumulative concentration–response curve. Three successively increasing concentrations of antagonist were equilibrated with each rat vas deferens; the antagonist was washed out before the addition of the next higher concentration. In another series of experiments, cumulative log concentration–response curves for bradykinin were constructed before and after co-incubation of NPC 349 (30 and 100 μM) and Hoe 140 (30 nM).

To investigate the specificity of NPC 349 (100 μM) and Hoe 140 (100 nM), neurogenic and musculotropic response curves were constructed to noradrenaline, angiotensin II and U46619 (0.01–100 μM) before and after incubation with the antagonists. Antagonist washout experiments were performed by constructing cumulative concentration–response curves to bradykinin before and after the addition of a high concentration of either NPC 349 (100 μM) or Hoe 140 (100 nM).

2.4. Data analysis

Increases in basal muscle tension (musculotropic response) are expressed as a percentage of the maximal response that was attained by bradykinin. The potentiation of the electrically-driven twitch (neurogenic response) is quantified as the percentage of the maximal bradykinin-induced increase in twitch over basal twitch height. EC_{50} values (the concentration of bradykinin required to cause half maximal response) and 95% confidence limits were calculated for neurogenic and musculotropic responses to bradykinin.

pA_2 values were derived from Schild plots using a curve fitting program (Baspak, GlaxoWellcome). As incubation with Hoe 140 decreased the maximal bradykinin musculotropic response (insurmountable antagonism), it was not possible to use conventional Schild analysis to determine the potency of the antagonist. Nevertheless, an apparent pK_B for Hoe 140 was derived using a double regression plot (Kenakin, 1984). Essentially, such a plot involves plotting $1/A$ vs. $1/A'$ where A and A' are the equieffective concentrations of agonist in the absence and presence of Hoe 140, respectively. An estimated pK_B is then derived by using the gradient (G) of this plot in the Gaddum Equation ($pK_B = -\log([B]/G - 1)$), where B is the antagonist concentration).

Experimental values are given as the mean ± S.E.M. for n vasa deferentia used.

2.5. Drugs and chemicals

Drugs used were: bradykinin {Sigma Chemical, Dorset}, NPC 349 (Bachem, UK), angiotensin II (NOVA Biochem, UK), Hoe 140 (synthesised by GlaxoWellcome, UK) (−)-noradrenaline bitartrate (Sigma Chemical, Dorset), and 9,11-deoxy-9α,11α-methanoepoxy prostaglandin $F_{2\alpha}$ (U46619, Upjohn, Kalamazoo, MI, USA). The chemicals for the physiological salt solution were of Analar grade and obtained from BDH, UK.

3. Results

3.1. Neurogenic and musculotropic effects of bradykinin in the vas deferens

Cumulative additions of bradykinin (1–3000 nM) to the electrically-stimulated rat vas deferens produced two distinct effects; a potentiation in the magnitude of the electrically-driven twitch (neurogenic response) and an increase in the basal tension of the muscle (musculotropic response). The mean EC_{50} values (nM) and 95% confidence limits for the initial bradykinin neurogenic and musculotropic curves ($n = 14$) were 109 (32–340) and 93 (28–345), respectively. The initial bradykinin neurogenic and musculotropic EC_{50} values were not significantly different from

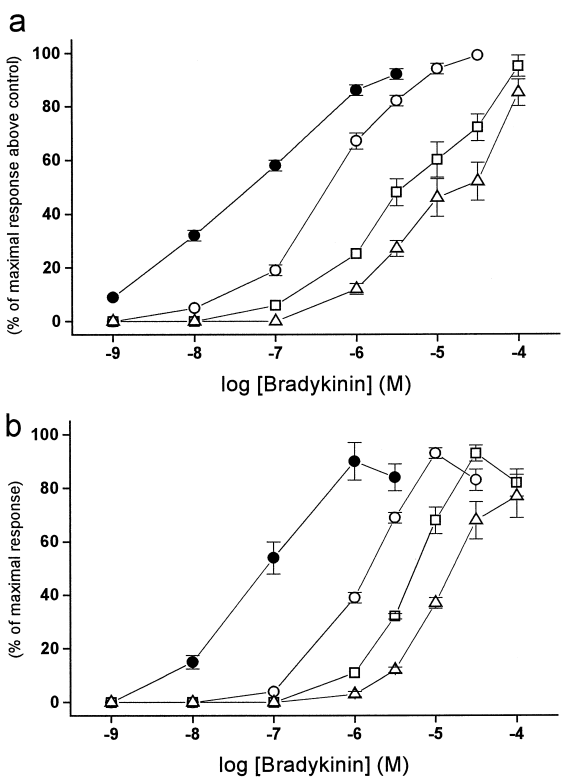

Fig. 1. Effects of NPC 349 (○ 10, □ 30 and △ 100 μM) incubated for 20 min on the control (●) neurogenic (a) and musculotropic (b) cumulative log concentration–response curves to bradykinin, added cumulatively. Each point is the mean ± S.E.M. (vertical bars) from seven separate experiments.

their respective EC_{50} values obtained in the three subsequent bradykinin cumulative neurogenic and musculotropic response curves ($P > 0.05$, paired *t*-test). No significant difference was found between the EC_{50} value for the bradykinin neurogenic response and that for the bradykinin musculotropic response ($P > 0.05$, paired *t*-test, $n = 30$).

3.2. Effects of NPC 349 and Hoe 140 on bradykinin neurogenic and musculotropic responses

The bradykinin B_2 receptor antagonists NPC 349 and Hoe 140 both produced concentration-dependent rightward shifts of the cumulative neurogenic and musculotropic response curves to bradykinin, although Hoe 140 was approximately two to three orders of magnitude more potent. NPC 349 (10–100 μM) produced surmountable antagonism of both the bradykinin neurogenic (pA_2 5.8 ± 0.1, Fig. 1a) and musculotropic (pA_2 6.3 ± 0.2, Fig. 1b) responses. A differing profile of antagonism was produced by Hoe 140 (10–100 nM), which caused surmountable antagonism of the bradykinin neurogenic response curve (pA_2 8.5 ± 0.5, Fig. 2a), but a concentration-related rightward shift and suppression of the maximal response of the bradykinin musculotropic response curve (apparent pK_B of 9.0 ± 0.2 with 30 nM Hoe 140, Fig. 2b).

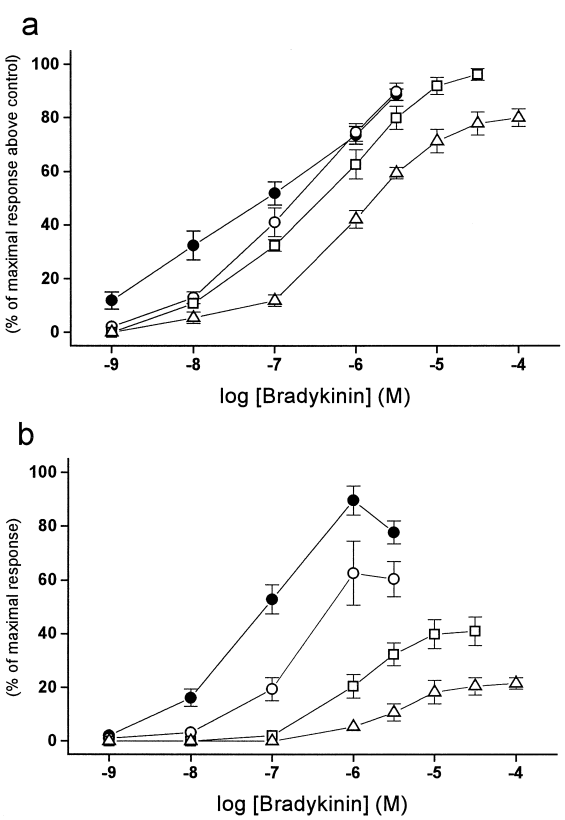

Fig. 2. Effects of Hoe 140 (○ 10, □ 30 and △ 100 nM) incubated for 20 min on the control (●) bradykinin neurogenic (a) and musculotropic (b) cumulative concentration–response curves. Each point is the mean ± S.E.M. (vertical bars) from eight separate experiments.

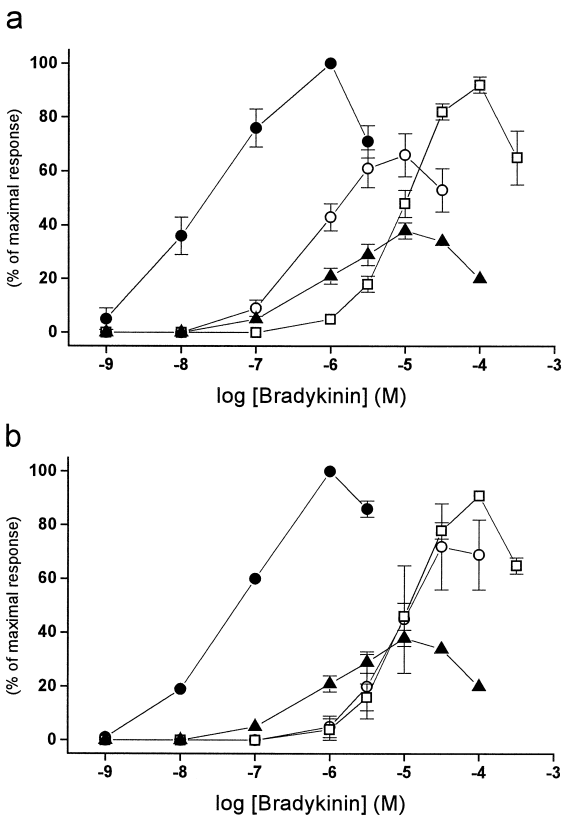

Fig. 3. Cumulative concentration–response curves for the musculotropic response to bradykinin in the absence (●) and in the presence of Hoe 140 (30 nM, 20 min incubation) alone (▲), or (a) co-incubated with NPC 349 (○ 30 and □ 100 μM) for the final 18 min of the 20 min incubation period and (b) co-incubated with NPC 349 (100 μM) for the final 2 (○) or 18 (□) min of the 20 min incubation period. Each point is the mean ± S.E.M. (vertical bars) from five individual experiments.

Neither NPC 349 (10–100 μM) nor Hoe 140 (10–100 nM) had any direct effect on the basal electrically-induced twitch response or on baseline tension ($P > 0.05$, paired *t*-test).

3.3. Effects of NPC 349 on Hoe 140-induced insurmountable antagonism of the bradykinin musculotropic response

Incubation (20 min) with Hoe 140 (30 nM) caused a rightward shift and approximately 70% suppression of the maximal response of the bradykinin musculotropic concentration–response curve (Fig. 3a). Increasing the incubation time of Hoe 140 (30 nM, 2–180 min) had no significant effect on the rightward shift or on the suppression of the maximal response of the bradykinin musculotropic curve ($P > 0.05$, un-paired *t*-test, $n = 6$). Co-incubation of NPC 349 (30 and 100 μM) for the final 18 min of the Hoe 140 (30 nM) incubation period (20 min) produced bradykinin musculotropic response curves which were displaced up-

wards in a concentration-related manner, and shifted to the left (NPC 349, 30 μM) or to the right (NPC 349, 100 μM) as compared to the bradykinin curve in the presence of Hoe 140 (30 nM) alone (Fig. 3a). In another related series of experiments, varying the incubation time (2 or 18 min) of a single concentration of NPC 349 (100 μM) in the presence of Hoe 140 produced no differences in the subsequent bradykinin musculotropic curves where there were shifts to the right and upwards displacements of the curves as compared to the bradykinin curve in the presence of Hoe 140 alone (Fig. 3b).

3.4. Specificity and wash-out experiments with NPC 349 and Hoe 140

The specificities of action of NPC 349 (100 μM) and Hoe 140 (100 nM) as bradykinin receptor antagonists were tested against the neurogenic and musculotropic responses to noradrenaline, angiotensin II, and the thromboxane A_2 mimetic, U46619 (0.01–100 μM). Neither the neurogenic nor the musculotropic cumulative concentration–response curves obtained to any of these agonists was affected by either NPC 349 or Hoe 140 ($n = 6$, $P > 0.05$, data not shown).

The effects of both NPC 349 and Hoe 140 (100 nM, 20–180 min incubation for Hoe 140 and 100 μM, 20 min incubation for NPC 349) could be fully reversed on washing with drug-free Krebs, since the bradykinin-induced neurogenic and musculotropic response curves obtained after antagonist wash-out were similar to the initial curves without antagonist ($n = 6$, $P > 0.05$, data not shown).

4. Discussion

The present study has demonstrated that addition of bradykinin to the electrically-stimulated rat vas deferens enhances the electrically-evoked twitch response (neurogenic response) and increases the basal muscle tension (musculotropic response). NPC 349 competitively antagonised both the neurogenic and the musculotropic responses caused by bradykinin, which is consistent with the findings of Rifo et al. (1987). Hoe 140 also caused surmountable antagonism of the neurogenic response but in striking contrast to NPC349, the antagonism of the musculotropic response to bradykinin was insurmountable. Insurmountable antagonism with Hoe 140 has previously been observed in other tissue preparations (see Section 1). Interestingly, Cuthbert et al. (1992) reported that the ability of the bradykinin analogue Lys-bradykinin to raise intracellular calcium was blocked in an insurmountable fashion by Hoe 140, although the blockade of bradykinin-induced electrogenic chloride secretion was surmountable.

There are several mechanisms that could explain the present findings of an insurmountable action of Hoe 140 on the bradykinin-induced musculotropic response curve in the rat vas deferens. Hoe 140 may act as (1) a 'non-specific' antagonist, (2) an irreversible antagonist, (3) an allosteric antagonist, (4) an antagonist specific for a subtype of the bradykinin B_2 receptor, or (5) a pseudo-irreversible antagonist. These various possible mechanisms are discussed below.

(1) The possibility that Hoe 140 is a 'non-specific' antagonist such that it could block the chain of events subsequent to receptor activation that lead to the production of a response, rather than the bradykinin B_2 receptor itself, is unlikely. Hoe 140 did not affect the neurogenic or the musculotropic responses induced by a range of agonists (angiotensin II, noradrenaline, and U46619) that evoked similar effects to bradykinin on this tissue, but through distinct receptors.

(2) Irreversible antagonism occurs when the antagonist-receptor interaction involves irreversible binding, and once the available receptor number has fallen to a level below that necessary to elicit a full agonist effect insurmountable antagonism is observed. This explanation is unlikely to account for the insurmountable antagonism of Hoe 140, since there is no evidence of irreversibility; the effects of the antagonist were readily reversed by washing the tissue, despite antagonist incubation times of up to 180 min. Furthermore, increasing the incubation time for Hoe 140 from 2 to 180 min did not result in any increase in the suppression of the maximal response. From these two findings, it is clear that Hoe 140 does not bind irreversibly with the bradykinin receptor and reaches equilibrium rapidly (< 2 min). Finally, the finding that the suppression in the maximal response induced by Hoe 140 could be reversed by co-incubation with the bradykinin B_2 receptor competitive antagonist NPC 349 (Fig. 3) provides further evidence against Hoe 140 being an irreversible antagonist.

(3) Allosteric modulation of the bradykinin B_2 receptor by Hoe 140 could be advanced to explain the insurmountable antagonism of the bradykinin musculotropic response. Essentially, allosteric antagonists act at a site distinct from the agonist binding site, and while not directly competing with the agonist for binding, serve to diminish (by changes in receptor affinity) the ability of the agonist-receptor complex to generate a response (Kaumann and Frenken 1985; Tucek and Proska, 1995). Invoking the theory of allosteric modulation to account for the present results fails to explain how the Hoe 140-induced depression of musculotropic activity of bradykinin can be reversed by concentrations of NPC 349 which also cause progressive rightward parallel shifts of bradykinin concentration–effect curves. It also fails to explain the difference in antagonist profile of Hoe 140 against the musculotropic and neurogenic effects of bradykinin.

(4) The difference between the antagonism of the neurogenic and musculotropic components of the tissue's response to bradykinin seen with Hoe 140 could be explained by the presence of different subtypes of the

bradykinin B_2 receptor. The possibility of bradykinin B_2 receptor subtypes (see Regoli et al., 1993) has been suggested in various preparations including vas deferens (Llona et al., 1987; Rifo et al., 1987), rat myometrial membranes (Liebmann et al., 1991), mouse neurobalstoma cells (Braas et al., 1988), and sheep femoral artery (Félétou et al., 1994). However, the existence of bradykinin B_2 receptor subtypes is controversial, as other investigations have failed to support their existence (Eggerickx et al., 1992; Pruneau et al., 1995; Miyamoto et al., 1999). The controversy has arisen partly as a consequence of using 'first' generation bradykinin receptor antagonists which have a low potency, and in some cases affinity for bradykinin B_1 receptors, following degradation. In addition, bradykinin B_2 receptor antagonists, including Hoe 140, show partial agonist activity in some preparations (Farmer and Burch, 1992; Félétou et al., 1994). There is no convincing genetic evidence for the hypothetical subtypes of bradykinin B_2 receptor in any species, and southern blot analysis suggests that there is only one gene closely related to the bradykinin B_2 receptor in each genome (McEachern et al., 1991; McIntyre et al., 1993). Reports of bradykinin B_2 receptor subtypes may simply reflect species differences in a single bradykinin B_2 receptor gene (Hess et al., 1994).

(5) Insurmountable antagonism of the bradykinin musculotropic response may be caused by Hoe 140 acting as a 'pseudo-irreversible' antagonist, i.e. being only slowly reversible from the bradykinin B_2 receptor. Pseudo-irreversibility results when agonist and antagonist compete directly for association with the receptor but the antagonist, by virtue of a very low off-rate (k_{off}) from the receptor, dissociates from it so slowly that it reduces the ability of the agonist to achieve its 'true' equilibrium with the receptor. This makes it appear that the antagonist has bound to the receptor irreversibly (Robertson et al., 1994). The proposal that Hoe 140 might be a pseudo-irreversible antagonist is supported by the results of the co-incubation experiments with Hoe 140 and NPC 349. Thus, when Hoe 140 dissociates from the bradykinin B_2 receptor the vacated receptor can be occupied by NPC 349, and the added bradykinin may then compete for bradykinin B_2 receptors with NPC 349 rather than Hoe 140. Since bradykinin can surmount the antagonism by NPC 349, the maximal response attainable by bradykinin increases in relation to the number of receptors occupied by NPC 349.

However, despite the apparent consistency of the antagonist profile of Hoe 140 in its antagonism of bradykinin-induced musculotropic effects in rat vas deferens with the pseudo-irreversible profiles previously reported for antagonism of angiotensin by GR117289 (Robertson et al., 1992), and indeed of 5-HT by various 5-HT antagonists (Bond et al., 1989; Prins et al., 1997), there is one crucial difference. Previous examples of pseudo-irreversible antagonists have all been characterised by slow rates of association (k_{on}) and dissociation (k_{off}) with their respective receptors. In the case of Hoe 140, there is nothing to suggest such slow receptor kinetics. The fact that the antagonist effects of Hoe 140 appeared to be maximal at 2 min, and that its effects were reversed by NPC 349 within 2 min, or by washing, suggests rather rapid kinetics. Our results are therefore inconsistent with the explanation for pseudo-irreversibility previously proposed by Robertson et al. (1994). In the light of this inconsistency, we suggest an alternative explanation consistent with our observations, which involves extending the two-state theory of receptor activity to include another inactive form of the receptor. There is an increasing body of evidence that the two-state theory with a thermodynamically stable inactive form of the receptor (R) for which antagonists and inverse agonists have a high affinity, and a thermodynamically less stable active form (R^*) for which agonists have a high affinity, is over-simplistic (Clark et al., 1999; Onaran and Gürdal, 1999). Furthermore, G-protein-coupled receptors may exist in a number of different states (Khorana, 1992) with different affinities for ligands (Krumins and Barber, 1997). In the model that we propose, there exists three states of the bradykinin receptor: a thermodynamically favourable (inactive) form (R), a thermodynamically reversible (activated) form (R^*), and a third thermodynamically unstable (inactivated) form ($R^\#$). The resting equilibrium between them may be represented as:

$$R^* \rightleftharpoons R \leftrightharpoons R^\#.$$

Within such a system, bradykinin and NPC 349 simply shift the equilibrium between R^* and R in a 'classical' fashion. However, if $R^\#$ is in unfavourable equilibrium with R, and for which Hoe 140 has high affinity, the presence of this compound would tend to shift the equilibrium in favour of this form at the expense of both R and more significantly R^*. Thus, the presence of Hoe 140 would favour receptors being in a form that is in direct equilibrium with R but not with R^* and therefore the ability of bradykinin to increase R^* to the level required to produce an agonist effect would be reduced. However, addition of a 'classical' antagonist such as NPC 349 would shift the equilibrium away from $R^\#$ towards R, a form which is in direct equilibrium with R^*, resulting in the renewed ability of bradykinin to cause a shift in equilibrium towards R^*. Such an explanation could account for the insurmountable nature of the antagonism of bradykinin by Hoe 140, but the reassertion of surmountability on the addition of NPC 349. The one remaining question is whether the theory can explain the fact that while the interaction between bradykinin and Hoe 140 is insurmountable on musculotropic effects, it is surmountable on neurogenic effects. These observations would be consistent with such a theory if there are differences in receptor reserve for the two different responses. Insurmountable antagonists cause parallel rightward shifts of agonist log concentration–response curves as long as there is a receptor reserve, but elimination of that reserve results in de-

pression of the maximum response. The highest concentration of Hoe 140 depressed the maximally-effective neurogenic responses to bradykinin (Fig. 2a), which may reflect an elimination of the receptor reserve. While our tentative theory provides a theoretical explanation for the experimental findings, further studies are needed to provide definitive evidence that such a process does account for the observed interaction between bradykinin, Hoe 140, NPC 349, and bradykinin B_2 receptors in the current study. If this theory is correct, it could also account for the pseudo-irreversible antagonism previously reported for antagonists at angiotensin and 5-HT-receptors (Bond et al., 1989; Robertson et al., 1994; Prins et al., 1997).

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