A new generation of bradykinin antagonists

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Abstract

Bradykinin B2 receptors are constitutively expressed, and require the entire peptide chain of bradykinin for recognition. Expression of B1 receptors is induced in inflammation; they recognize BK-(1-8). Hereofore blockade of all the actions of bradykinin required two different antagonists, one for each class of receptors. The new antagonists described here are full chain antagonists having high potency on B2 receptors, but they are also very potent antagonists for B1 receptors. They are highly resistant to kininases and show very long action in vivo. These antagonists contain the novel amino acid α-(2-indanyl)glycine (Igl) at positions 5 and 7. The peptide dArg-Arg-Pro-Hyp-Gly-Igl-Ser-dIgl-Oic-Arg (designated B9430) shows all these desirable characteristics. It represents a new class of bradykinin antagonist peptides.

Keywords: Blood pressure; Bradykinin; Bradykinin antagonist; Bradykinin receptor

1. Introduction

A decade of animal studies and recent clinical trials have indicated that bradykinin (BK) antagonists may become important drugs for anti-inflammatory medicine. Antagonists for BK B2 receptors were introduced in 1984, (Vavrek and Stewart, 1985) and proved to be the necessary tools that allowed demonstration of participation of BK in regulation of every major physiological system and initiation or mediation of much pathophysiology (Stewart, 1989). Binding to B2 receptors requires the full BK chain, including the C-terminal arginine. Although B1 antagonists had been described in 1977 (Regoli et al., 1977), they did not attract much interest until the recent demonstration that B1 receptors, normally not present in most tissues, are expressed in chronic inflammation (Perkins et al., 1993; Marceau, 1995). The physiological agonists for B1 receptors are BK(1-8) and Lys-BK(1-8), that arise from action of carboxypeptidase N on BK and kallidin. Replacement of the C-terminal phenylalanine in these peptides by a hydrophobic aliphatic amino acid yielded the first B1 antagonists. The earliest antagonists for both receptor classes were not very potent, and were rapidly degraded in vivo (Stewart and Vavrek, 1991). Continued development, especially of B2 antagonists, yielded a second generation of antagonists that are potent and durable enough to show activity in humans.
Table 1
Structures of bradykinin and early antagonists

<p>| | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Bradykinin:</td>
<td>Arg-Pro-Gly-Phe-Ser-Pro-Phe-Arg</td>
</tr>
<tr>
<td>NPC-349:</td>
<td>dArg-Arg-Pro-Hyp-Gly-Thi-Ser-Phe-Thi-Arg</td>
</tr>
<tr>
<td>HOE-140:</td>
<td>dArg-Arg-Pro-Hyp-Gly-Thi-Ser-oTic-Oic-Arg</td>
</tr>
<tr>
<td>B-8838:</td>
<td>dArg-Arg-Pro-Hyp-Gly-Cpg-Ser-dCpg-Cpg-Arg</td>
</tr>
<tr>
<td>NPC-17731:</td>
<td>dArg-Arg-Pro-Hyp-Gly-Thi-Ser-oHype-Oic-Arg</td>
</tr>
<tr>
<td>CP-0127:</td>
<td>dArg-Arg-Pro-Hyp-Gly-Thi-Cys-dPhe-Leu-Arg</td>
</tr>
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Among the second generation B₂ antagonists the Hoechst Icatibant (HOE-140) (Hock et al., 1991; Wirth et al., 1991b) and the Cortech Bradycor (CP-0127) (Cheronis et al., 1992) have attracted most interest (see Table 1). In the first generation B₂ antagonists, such as the Stewart NPC-349, the d-amino acid residue at position seven blocked action of angiotensin converting enzyme (kininase II, ACE), the principal kininase of lung (Togo et al., 1989), and the N-terminal d-arginine residue blocked aminopeptidase action. These antagonists were still degraded by plasma kininase I (carboxypeptidase N, CPN) (Regoli et al., 1986a, b) and by the membrane-bound endopeptidase 3.4.24.11 (enkephalinase). Indeed, the first generation antagonists, such as NPC349, showed B₁ antagonist activity in vivo, due to enzymatic removal of the C-terminal arginine (Regoli et al., 1986a, b). The significant structural feature of Icatibant is the incorporation of imino acids, which greatly restrict conformation and inhibit enzyme action, at positions seven and eight. Incorporation of octahydroindolecarboxylic acid (Oic) at position eight made this peptide resistant to cleavage by CPN and thus greatly extended its in vivo activity. The bulky d-tetrahydroisoquinoline-carboxylic acid (Tic) at position seven, combined with Oic⁸, strongly restricts the conformational freedom of the important carboxyl end of the peptide to a shape evidently preferred by the receptors (Kyle et al., 1993). The very hydrophobic nature of these residues is probably also important, causing Icatibant to have a slow ‘on-time’ and a very long persistence at or near receptors. Bradycor owes its increased potency to its dimeric nature, with perhaps some additional contribution from the hydrophobic character of the linker moiety. Despite these improvements, both of these antagonists are slowly degraded by plasma and tissue extracts.

In continuing investigations in the Stewart laboratory, many novel amino acids have been incorporated into BK antagonists. Four years ago modification of NPC-349 to include α-cyclopentylglycine (Cpg) (Hill and Dunn, 1969) at positions five, seven and eight gave the first all-aliphatic BK antagonist (Vavrek et al., 1992). Chemists at Nova also synthesized an antagonist having an aliphatic residue at position seven (Kyle et al., 1991). The high potency and persistence in vivo of the Cpg antagonists suggested further investigation of amino acids having cyclic substituents. The most dramatic improvement came with introduction of α-(2-indanylglycine (Igl) (Porter and Shive, 1968) into the antagonist structure. B9340, in which d-Igl replaced the d-Tic at

Table 2
Structures of the bradykinin antagonists used in this study

<p>| | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Lys[Leu⁸]BK(1-8)</td>
<td>Lys-Arg-Pro-Gly-Phe-Ser-Pro-Leu</td>
</tr>
<tr>
<td>B9066</td>
<td>dArg-Arg-Pro-Hyp-Gly-Cpg-Ser-oTic-Cpg</td>
</tr>
<tr>
<td>B9812</td>
<td>dArg-Arg-Pro-Hyp-Gly-Igl-Ser-oTic-Oic</td>
</tr>
<tr>
<td>B9816</td>
<td>dArg-Arg-Pro-Hyp-Gly-Igl-Ser-oTic-Leu</td>
</tr>
<tr>
<td>B9838</td>
<td>Lys-Lys-Arg-Pro-Hyp-Gly-Igl-Ser-oTic-Oic</td>
</tr>
<tr>
<td>B9958</td>
<td>Lys-Lys-Arg-Pro-Hyp-Gly-Igl-Ser-oTic-Arg</td>
</tr>
<tr>
<td>[des-Arg⁹]-HOE-140:</td>
<td>dArg-Arg-Pro-Hyp-Gly-Thi-Ser-oTic-Oic</td>
</tr>
<tr>
<td>B9958</td>
<td>Lys-Lys-Arg-Pro-Hyp-Gly-Cpg-Ser-oTic-Cpg</td>
</tr>
<tr>
<td>HOE-140:</td>
<td>dArg-Arg-Pro-Hyp-Gly-Thi-Ser-oTic-Oic-Arg</td>
</tr>
<tr>
<td>B9430</td>
<td>dArg-Arg-Pro-Hyp-Gly-Thi-Ser-oTic-Oic-Arg</td>
</tr>
<tr>
<td>B9340</td>
<td>dArg-Arg-Pro-Hyp-Gly-Thi-Ser-oTic-Oic-Arg</td>
</tr>
<tr>
<td>B9668</td>
<td>Gun-dArg-Arg-Pro-Hyp-Gly-Thi-Ser-oTic-Oic-Arg</td>
</tr>
<tr>
<td>CP-0597</td>
<td>dArg-Arg-Pro-Hyp-Gly-Thi-Ser-oTic-NChg-Arg</td>
</tr>
</tbody>
</table>
position seven in Icatibant, is a very potent antagonist, but the most interesting peptide is B9430, which has L-Igl at position five, D-Igl at position seven, and Oic at position eight. This antagonist shows truly impressive high potency and long duration of action in vivo. The most remarkable property of the antagonists containing Igl is their high potency at B1 receptors in addition to the anticipated B2 activity. They are active at human B1 and B2 receptors. A single subcutaneous injection of B9430 in rabbits blocks the hypotensive action of bradykinin for more than 24 h.

2. Materials and methods

2.1. Materials

All peptides used were synthesized in the Department of Biochemistry, UCHSC or in the Department of Chemistry, Cortech Inc. The structures of the compounds used in these studies are shown in Table 2.

2.2. Peptide synthesis

Peptides were synthesized by the solid phase method, generally using Boc-amino acids and conventional side-chain blocking groups (Stewart and Young, 1984). Incorporation of the sterically hindered amino acids required use of efficient coupling agents, such as BOP-HOBt or HBTU. Peptides were purified by countercurrent distribution followed by preparative reversed-phase HPLC where needed. For exploratory incorporation of novel optically active amino acids, the racemic amino acid was incorporated into the peptide and the two diastereomeric peptides were separated by HPLC. For synthesis of larger amounts, the new amino acids were resolved enzymatically to optical homogeneity. Peptides were characterized by amino acid analysis, TLC, and laser desorption mass spectroscopy (LDMS). The ‘found molecular weight’ determined by amino acid analysis was used for accurate calculation of molar doses for biological experiments.

2.3. Synthesis of α-(2-indanyl)glycine (Igl)

Diethyl acetamidomalonate was alkylated with 2-bromoindane, using sodium ethoxide (Porter and Shive, 1968). Following alkaline and acid hydrolysis, the free D,L-amino acid was acetylated with acetic anhydride. Acetyl L-Igl was selectively hydrolyzed by hog kidney acylase I (Sigma). D-Igl was prepared by acid hydrolysis of the residual acetyl derivative following removal of the L-amino acid. The purified isomeric amino acids showed \( \left[ \alpha \right]_{D}^{22} = +35.4^\circ \ \text{ (c2, 2 N HCl)} \), and were converted to the Boc derivatives with Boc anhydride by the standard procedure; mp 88–90°C, \( \left[ \alpha \right]_{D}^{22} = +16.9^\circ \ \text{(C2, EtOH)} \).

2.4. Synthesis of N-(2-indanyl)glycine

2-Indanone was reductively alkylated by glycine methyl ester, using NaCNBH3. The ester was saponified with NaOH and the purified amino acid was converted to the Boc derivative by the standard method (Stewart and Young, 1984) to yield a crystalline solid, mp 130–131°C.

2.5. Synthesis of Boc-hexahydro-(2-indanyl)glycine (Boc Hig)

Boc-Igl was dissolved in ethanol and hydrogenated over Pd/C at 80°C and 100 atm to yield Boc-Hig, which was obtained as an oil.

2.6. In vitro functional assays

Assays on isolated ileum from guinea pigs starved overnight and isolated uterus from rats pretreated (16 h) with diethylstilbestrol were carried out by published procedures (Cheronis et al., 1992). Concentration-effect curves were constructed to BK in the absence and presence of the antagonist (15 min pre-incubation) and the potency at the B2 receptor was calculated as the pA2 (Arunlakshana and Schild, 1959). The isolated rabbit aorta was used as an assay to determine the B1 antagonist potency of the compounds using the method described previously (Cheronis et al., 1994). Potency values in this assay were expressed as the pIC50, which is the negative
logarithm of the dose of antagonist required to produce a 50% reversal of the sustained contraction to [des-Arg⁹]-BK on the aorta. Studies were performed on human ileum as described by Zuzack et al. (1996) for the determination of antagonist potency at the B₂ and the B₁ receptor. Compounds were pre-incubated with the tissues for 15 min. Antagonist potency at the B₂ receptor was calculated as the pA₂ and at the B₁ receptor as the pK₅b.

2.7. Receptor binding

Receptor binding studies were performed using the human cloned and expressed B₂ receptor and the human B₁ membrane receptor in IMR-90 cells as described by Burkard et al. (1996).

2.8. Rat blood pressure

Male rats were starved overnight and anesthetized with a mixture of diallylbarbituric acid, urethane and monomethyl urea or urethane alone (1.25 g/kg, i.p.). Blood pressure was recorded from a femoral artery. The right jugular vein and left carotid artery were cannulated for drug administration (Roblero et al., 1973). The potency, selectivity and duration of action of B9430 given by an intravenous infusion was assessed. Potency against BK-induced (40 pmol) hypotension were calculated following administration of B9430, 0.03–1.0 μg/kg per min for 15 min. After stopping the last dose infusion of B9430, BK was given at 15 min intervals for the remainder of the experiment to determine the duration of action. In separate experiments, the selectivity of B9430 was assessed against acetylcholine (20 nmol), norepinephrine (20 nmol), substance P (10 pmol) and angiotensin II (20 pmol).

2.9. Dog blood pressure

Dog blood pressure studies were performed to assess the B₁ and B₂ antagonist potency of the compounds, their selectivity and their duration of action as described by Hanson et al. (1996). Potency was calculated in terms of the ED₅₀, i.e., the infused dose of the compound producing a 50% reduction in the hypotensive response to the B₂ agonist, BK (1 nmol) or the B₁ agonist, [des-Arg⁹]-BK (25 nmol). Duration of action was assessed by repeated administration of the agonists following stopping the final dose infusion of the agonist which in each case was 1 μg/kg per min. Selectivity studies were performed with the highest dose infusion of the antagonists against acetylcholine (20 nmol), norepinephrine (20 nmol), substance P (10 pmol) and angiotensin II (10 pmol).

2.10. Rabbit blood pressure

Male New Zealand White rabbits (2–2.5 kg) were anesthetized with ketamine 35 (mg/kg, i.m.) and xylazine (5 mg/kg, i.m.) and prepared with femoral vein and arterial catheters for the administration of drugs and the recording of blood pressure, respectively. The effect of B9430 (30 μg/kg, s.c.) was studied. Three rabbits were also pretreated 20–22 h earlier with B9430 (30 μg/kg, s.c.).

2.11. Carrageenan paw edema and hyperalgesia

The effect of B9430 in the rat carrageenan paw edema and hyperalgesia test was studied. Paw volume and hyperalgesia were measured before and at hourly intervals over a 6-h period after an intraplantar injection of 1% carrageenan, using a Hugo Basil plethysmometer and a Hugo Basil algesiometer respectively.

2.12. Compound stability

Lung and kidney tissues were obtained and prepared as indicated (Booth and Kenny, 1974; Skidgel et al., 1984). Protein concentration was determined by the method of Bradford (1976). Tissue membrane samples were diluted in phosphate-buffered saline (PBS). Human plasma was prepared from heparinized whole blood by centrifugation. The various BK antagonists were prepared as 1 mM stock solutions in PBS. The compounds were diluted to 100 μM in the tissue samples and incubated at 37°C. At various times, reactions were stopped and samples were analyzed using reversed-phase HPLC (3.6–72%
Table 3
Relative potencies of selected compounds on rabbit aorta (B₁), rat uterus (B₂) and guinea pig ileum (B₂) in vitro. N = 3-6.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Rabbit aorta B₁</th>
<th>Rat uterus B₂</th>
<th>G-pig ileum B₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOE-140</td>
<td>i.a.</td>
<td>9.5</td>
<td></td>
</tr>
<tr>
<td>CP-0597</td>
<td>i.a.</td>
<td>9.5</td>
<td></td>
</tr>
<tr>
<td>dArg¹⁰Leu⁶KD</td>
<td>7.9</td>
<td>i.a.</td>
<td>i.a.</td>
</tr>
<tr>
<td>des-Arg⁹HOE-140</td>
<td>7.1</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td>B9340</td>
<td>6.9</td>
<td>8.9</td>
<td>8.6</td>
</tr>
<tr>
<td>B9430</td>
<td>6.5</td>
<td>9.9</td>
<td>8.6</td>
</tr>
<tr>
<td>B9066</td>
<td>8.1</td>
<td>5.3</td>
<td>i.a.</td>
</tr>
<tr>
<td>B9812</td>
<td>8.2</td>
<td>i.a.</td>
<td>i.a.</td>
</tr>
<tr>
<td>B9816</td>
<td>8.0</td>
<td>i.a.</td>
<td>i.a.</td>
</tr>
</tbody>
</table>

ₐ pIC₅₀.
ₚ pA₂.
i.a., inactive.

CH₃CN in 0.1% TFA). Compound half-life was determined by integration of peak areas from HPLC.

3. Results

3.1. In vitro functional studies

Results for assays of selected compounds on animal tissues in vitro are shown in Table 3. HOE-140 and CP-0597 were potent and selective B₂ antagonists with no antagonist activity at the rabbit aorta B₁ receptor. Lys-[Leu⁶]-BK(1-8) was a potent and selective B₁ antagonist, being inactive on the uterus and ileum. [Des-Arg⁹]-HOE-140 possessed B₁ antagonist activity and was a weak B₂ antagonist on the rat uterus. B9340 and B9430 were potent B₂ antagonists on both the rat uterus and guinea pig ileum and both possessed B₁ antagonist activity on the aorta. B9066, B9812 and B9816 were all potent and selective B₁ antagonists, being weak to inactive on uterus and ileum.

3.2. Human in vitro receptor studies

Comparison of the receptor binding and functional activities for the compounds on human B₁ and B₂ receptors is shown in Table 4. HOE-140 was a relatively selective and potent compound at the B₂ receptor, although it did possess weak B₁ receptor binding and antagonist activity. Lys-[Leu⁶]-BK(1-8) was found to be a potent B₁ antagonist ligand and was inactive at the B₂ receptor. B9430 and B9340 were potent antagonist ligands at both the B₂ and the B₁ receptors being a half log unit more potent than HOE-140 and only a log unit less potent than Lys-[Leu⁶]-BK(1-8) on the human ileum B₂ and B₁ receptors respectively. B9958, B9858, B9812, B9066 and B9816 were potent B₁ ligands with weak B₂ antagonist activity. B9958 and B9858 were 50-100 fold more potent than Lys-[Leu⁶]-BK(1-8) at the B₁ receptor and 100- and 10000-fold more potent at the B₁ compared to the B₂ receptor, respectively.

3.3. Rat blood pressure

B9430 was found to have an ED₅₀ of 0.17 ± 0.04 μg/kg per min (n = 6) against BK (40 pmol)-in-
Table 5
Comparative cardiovascular profile of BK antagonists at dog B₁ and B₂ receptors

<table>
<thead>
<tr>
<th>Compound</th>
<th>ED₅₀ a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B₂</td>
</tr>
<tr>
<td>HOE-140</td>
<td>0.02±0.01</td>
</tr>
<tr>
<td>CP-0597</td>
<td>0.03±0.02</td>
</tr>
<tr>
<td>Lys-[Leu⁸]BK(1-8)</td>
<td>Inactive</td>
</tr>
<tr>
<td>B9812</td>
<td>Inactive</td>
</tr>
<tr>
<td>B9858</td>
<td>Inactive</td>
</tr>
<tr>
<td>CP-0364</td>
<td>4.6±0.5</td>
</tr>
<tr>
<td>B9340</td>
<td>0.17±0.04</td>
</tr>
<tr>
<td>B9430</td>
<td>0.05±0.01</td>
</tr>
</tbody>
</table>

a Values are in μg/kg per min ± SEM; n = 3–4/group.

Reduced hypotension. After stopping the highest infusion dose used (1 μg/kg per min for 15 min), BK was administered at 15 min intervals and was found to be still almost completely blocked 3.5 h later (n = 6). This latter dose of B9430 had no effect on blood pressure responses to acetylcholine, substance P, norepinephrine or angiotensin II (n = 6).

3.4. Dog blood pressure

Several compounds were evaluated in the dog for potency at the B₁ and B₂ receptors and for selectivity and duration of action. The potency data are seen in Table 5. B9430 was found to have potency equivalent to that of HOE-140 and CP-0597 at the B₂ receptor and to Lys-[Leu⁸]-BK(1–8) at the B₁ receptor. B9812 and B9858 were selective antagonists at the B₂ receptor, being approx. 20 times more potent than Lys-[Leu⁸]-BK(1–8). B9430 was more potent at both the B₁ and B₂ receptors compared to the heterodimer B₁/B₂ antagonist, CP-0364. The duration of action of Lys-[Leu⁸]-BK(1–8) was only 15 min whereas all the other compounds had durations of action of at least 4 h. The duration of action of CP-0364 was not studied. All compounds were selective in that they did not antagonize blood pressure responses to acetylcholine, norepinephrine, substance P or angiotensin II; the effect of B9430 is shown in Fig. 1.

3.5. Rabbit blood pressure

Fig. 2 shows the effect of B9430 given s.c. at a dose of 30 μg/kg; within 5 min the response to BK was totally inhibited. In these experiments (n = 3) the effect lasted for the duration of the experiment (6 h). When rabbits (n = 3) were pretreated with 30 μg/kg s.c. B9430 and 20–22 h later anesthetized and prepared for blood pressure recording, the hypotensive effect of BK, but not of acetylcholine, was totally inhibited 24 h after administration of the antagonist (Fig. 2).
3.6. Carrageenan paw edema / hyperalgesia

B9430 produced significant inhibition of carrageenan paw edema and hyperalgesia. B9430 was equipotent with HOE-140 against hyperalgesia (Fig. 3). Against edema, HOE-140 at 1 mg/kg s.c. inhibited the response only during the first 3 h, whereas B9430 at the same dose produced significant inhibition over the whole 6-h study period (Fig. 4).

3.7. Compound stability studies

The half-lives in rat kidney brush-border, rat lung membrane and human plasma for Lys-[Leu\(^{5}\)]-BK(1–8) were 0.03, 0.09 and 0.44 h respectively, whereas those for HOE-140, CP-0597, B9430, and B9812 were > > 6 h.

4. Discussion

We have described the pharmacological profile of a unique class of bradykinin antagonist peptides containing indanylglycine or cyclopentylglycine. Some of these compounds (e.g., B9430) possessed potent antagonist activity, in vitro and in vivo, at both B\(_1\) and B\(_2\) receptors, whereas others (B9858 and B9958) were highly potent and selective B\(_1\) antagonists. B9430 is unique in its pharmacological profile, since it is a potent antagonist at both B\(_1\) and B\(_2\) receptors but it still retains the C-terminal arginine residue. Until now, an absolute requirement for good binding and function of an agonist or antagonist at the B\(_1\) receptor has been the absence of a C-terminal arginine group. It has been demonstrated that BK and Lys-BK can be metabolized by carboxypeptidases into their respective des-Arg\(^9\) derivatives, converting the parent B\(_2\) receptor agonist into potent and selective B\(_1\) receptor agonists (Regoli and Barabé, 1980; Regoli et al., 1986a, b). An equivalent conversion of B\(_2\) antagonists into B\(_1\) antagonists can also be achieved via this type of metabolism (Regoli et al., 1986a). The dual activity of B9430 at both B\(_1\) and B\(_2\) receptors cannot be accounted for by metabolism via carboxypeptidases, as this compound is extremely stable to most peptidases. This is in stark contrast to HOE-140, another stable peptide which is a highly potent and selective B\(_2\) antagonist (Hock et al., 1991; Wirth et al., 1991b; Lembeck et al., 1991). The only difference between B9340, B9430 and HOE-140 is the presence of D-Igl in place of D-Tic in position 7 and Igl in place of Thi in position 5. This seemingly minor change confers B\(_1\) antagonist activity on the compound in addition to retaining B\(_2\) binding and antagonist functional activity. It is possible that Igl in position 7 is allowing more conformational rotation than D-Tic, allowing it to access both the B\(_2\) and the B\(_1\) binding pockets.
The fact that this can be achieved with the terminal arginine present is difficult to explain. HOE-140 is predominantly a selective B2 antagonist, although on bovine aorta endothelial cells it has been demonstrated to inhibit B1 receptor mediated production of cGMP (Wiener and Wirth, 1992). We also found weak B1 binding and function in this study, but HOE-140 was 100–1000 times less potent than Lys-[Leu8]-BK(1–8).

As with other B2 antagonists, removal of the C-terminal arginine from these new peptides yielded B1 antagonists. In contrast to previous compounds, the des-Arg derivatives of B9430 were more potent than the standard B1 antagonist Lys-[Leu8]-BK(1–8). This high B1 antagonist potency was also achieved with cyclopentylglycine analogs of [des-Arg9-HOE-140 (B9066)]. Even greater potency and selectivity for the B1 receptor was achieved by classical N-terminal extension of the des-Arg9-derivatives with the positively charged amino acid lysine (B9858, B9958). This is consistent with previous structure–activity studies with B1 antagonist ligands (Regoli and Barabé, 1980). Des-Arg9-HOE-140 has been described as a potent B2 antagonist (Wirth et al., 1991a), but in contrast to the derivatives of B9430, we found this antagonist to be almost 10-fold weaker on the human B1 receptors than Lys-[Leu8]-BK(1–8).

These novel compounds possessed an impressive pharmacological profile in vivo. B9430 was found to be a potent, long-acting, selective antagonist of B2 receptor-mediated hypotension in the rat. Following a short intravenous infusion of a low dose the duration of action was at least 4 h. This was confirmed in the rabbit and the dog. In the rabbit a low dose, given s.c. produced a rapid, selective inhibition of the BK-induced hypotension which lasted for at least 6 h. In a second set of experiments in which animals were pretreated with B9430 at 30 μg/kg s.c., the BK antagonist effect was still present 24 h later. The rapid onset of action and the long duration of action of this low dose of the compound would suggest that B9430 or a similar compound could be suitable for the rapid relief of pain or other symptoms in conditions where bradykinin is believed to be involved; one such area which should be considered is migraine.

The dog experiments highlighted the unique B1/B2 antagonist profile of B9430 as compared to HOE-140 and CP-0597, highly potent B2 antagonists (Hock et al., 1991; Wirth et al., 1991b; Lembeck et al., 1991; Goodfellow et al., 1996), to the potent B1 antagonist, Lys-[Leu8]-BK(1–8) (Regoli and Barabé, 1980) and to the heterodimeric B1/B2 antagonist CP-0364 (Cheronis et al., 1994). B9430 was the most potent compound at both receptors and had a long duration of action. B9858 was shown to be a highly potent and selective antagonist at the B1 receptor in the dog, with a long duration of action. Compounds such as B9858 represent a new class of compounds which are potent, stable, selective B1 antagonists that will be useful pharmacological tools and potential therapeutics. This compound was 50 times more potent than Lys-[Leu8]-BK(1–8) in the dog, and the latter compound was short-acting, having a duration of action of only 15 min compared to at least 4 h for B9858.

B9430 was tested in one model of inflammation, the rat carrageenan paw edema and hyperalgesia and compared to HOE-140. On a dose weight basis, B9430 and HOE-140 were equipotent analgesic agents. However, against paw edema, HOE-140 at a dose of 1 mg/kg inhibited the edema response only during the first 3 h of the experiment. In contrast, B9430 at the same dose produced significant inhibition of edema over the entire 6 h study period. It is possible that the early phase of the edema response involves B2 receptors, whereas during the later phases, B1 receptors are upregulated and become functional. Although further studies are required, this hypothesis is consistent with numerous previous studies which have demonstrated early B2 and later B1 receptor involvement in inflammatory models (Marceau et al., 1980, 1983, 1984; Perkins et al., 1993; Perkins and Kelly, 1993; Siebeck et al., 1989).

In conclusion, we have reported the pharmacological profile of a novel class of compounds containing, in particular, indanylglycine. Substitution of this amino acid for d-Tic and Thi in HOE-140 conferred unexpected potent B1 and B2 antagonist activity on the compound. In addition, highly potent and selective, stable B1 receptor antagonists were achieved by synthesizing [des-Arg9] derivatives of these compounds and extending the N-terminus with lysine. The pharmacological characteristics of compounds such as B9430 and B9858, being highly potent, selective, stable and very long acting, would suggest
that these compounds will be not only useful pharmacological tools but also potential therapeutics in pathological conditions in which both B_2 and B_1 or only B_1 receptors are involved.

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**References**


Burkard M, Zuzack JS, Jones S, Francis M, Whalley ET, Stewart JM, Gera L. Comparative profile of novel potent bradykinin antagonists at human B_1 and B_2 receptors. Immunopharmacology 33, in press.


Stewart JM, Vavrek RJ. Chemistry of peptide B_2 bradykinin