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Bradykinin analogues: differential agonist and antagonist activities suggesting multiple receptors

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Bradykinin analogues with specific antagonist activity in several bioassays were evaluated for effects on [^{3}H]-bradykinin receptor binding sites and inositol phosphate production in neuroblastoma N1E-115 cells. The analogues varied in their affinities for bradykinin receptors in guinea-pig ileum and N1E-115 cell membranes, in their effects on uterine and ileal contractions and in their agonist or antagonist activity on phosphoinositide turnover in N1E-115 cells. These tissue specific effects suggest the presence of multiple bradykinin receptor subtypes.

Introduction The nonapeptide bradykinin (Bk) (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) influences inflammation, pain, and cardiovascular and gastrointestinal function. Several Bk derivatives are potent and specific Bk antagonists in guinea-pig ileum and rat uterus smooth muscle assays, and in the rat blood pressure kinin assay (Vavrek & Stewart, 1985a, b; Stewart & Vavrek, 1986). We have examined effects of these derivatives on Bk receptor binding and phosphoinositide (PI) turnover in neuroblastoma cells possessing functional Bk receptors (Snider & Richelson, 1984; Yano *et al.*, 1985). We describe tissue specific differential agonist and antagonist activities of the Bk derivatives, suggesting a multiplicity of Bk receptors.

Methods Murine neuroblastoma cells (clone N1E-115, passages 15–25) were cultured in Dulbecco's modified Eagle's medium (Grand Island Biological Company) without antibiotics containing 10% newborn calf serum as described previously (Braas *et al.*, 1983; Snider & Richelson, 1984).

N1E-115 neuroblastoma cells were harvested, homogenized in 5 mM Tris-HCl, pH 7.4, containing 0.32 M sucrose in a Teflon-glass homogenizer, and centrifuged at 600 *g* for 10 min. Guinea-pig ileum was homogenized in 25 mM trimethylaminoethanesulphonic acid (TES), pH 6.8, containing 1 mM 1,10-phenanthroline. The N1E-115 cell supernatant and ileum homogenate were centrifuged at 50000 *g* for 10 min; the pellets were washed, centrifuged, and resuspended in assay buffer consisting of 25 mM TES, pH 6.8, containing 1 mM 1,10-phenanthroline, 140 μg ml^{-1} bacitracin, 1 mM dithiothreitol, and 0.1% bovine serum albumen (BSA). Incubations containing 25–50 μg of membrane protein and 50 pM [^{3}H]-Bk (Amersham, 61 Ci mmol^{-1}) were conducted for 90 min at 25°C in a final volume of 2 ml, and terminated by rapid filtration through Whatman GF/B glass fibre filters (Manning *et al.*, 1986). Specific saturable [^{3}H]-Bk binding was calculated as total binding less nonspecific binding, determined with 50 nM unlabelled Bk (Peninsula Laboratories, Inc.).

To assay PI turnover, monolayers of N1E-115 cells were prelabelled with myo-[^{3}H]-inositol (Amersham, 13.8 Ci mmol^{-1}) for 24 h, washed, and harvested with Krebs–Ringer bicarbonate buffer containing 10 mM glucose (KRB). Cells (250000 per tube) were incubated at 37°C in KRB containing 140 μg ml^{-1} bacitracin, 1 μM SQ20881 (Peninsula Laboratories, Inc., an inhibitor of Bk degradation), and 10 mM LiCl for 10 min before incubation with Bk or Bk analogue for 5 min. Incubations were terminated with chloroform : methanol (1 : 2 v/v) and the aqueous phase assayed for total inositol phosphate (Berridge *et al.*, 1982; 1983).

Results Although less potent than Bk itself, several derivatives competed for [^{3}H]-Bk binding in the low nanomolar range (Table 1). Differences in receptor recognition sites between guinea-pig ileum and N1E-115 neuroblastoma cells were observed. Amino terminal extended Bk derivatives had 10 fold greater potency in N1E-115 cell than ileal membranes despite similar potencies of Bk and other Bk analogues in the two systems.

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Table 1 Bradykinin analogue effects on bradykinin-stimulated phosphoinositide turnover and [^{3}H]-bradykinin binding

*Analogue**	*Inhibition of phosphoinositide turnover* IC_{50} (μM)	*Inhibition of smooth muscle contraction* *GPI* pA_2	*Rat uterus* pA_2	*[^{3}H]-bradykinin competing potency* *GPI* K_i (nM)	*N1E-115* K_i (nM)
Bradykinin (Bk)	AG 100%	AG 100%	AG 100%	0.01	0.01
[D-Phe^7]-Bk	AG 130%	5.0	AG 1%	8.3	1.5
[Thi^{5,8}, D-Phe^7]-Bk	AG 90%	6.3	6.4	3.5	1.6
[Hyp^3, Thi^{5,8}, D-Phe^7]-Bk	0.55	5.7	–	18	2.3
Lys-Lys[Thi^{5,8}, D-Phe^7]-Bk	0.32	5.3	6.0	0.60	0.07
D-Arg[Hyp^3, Thi^{5,8}, D-Phe^7]-Bk	0.14	6.3	–	0.85	0.05
D-Arg[Hyp^{2,3}, Thi^{5,8}, D-Phe^7]-Bk	0.10	6.0	–	1.3	0.13
[des-Arg^9]-Bk	No effect	AG 1%	AG 1%	>1000	>1000

Phosphoinositide turnover assays were performed as described in the text. Agonist activity (AG) at 1 μM is given as percentage of Bk potency at 1 μM. Antagonist activity is given as IC_{50} (μM) values in the presence of 1 μM Bk. Antagonism of the Bk-induced myotropic response of guinea-pig ileum (GPI) and rat uterus by Bk analogues are from Stewart & Vavrek (1986), Vavrek & Stewart (1985a, b) and Steward (1971). Data are given as antagonist potency (pA_2 value) or as agonist activity (AG). Displacement of specifically bound [^{3}H]-Bk to guinea-pig ileum (GPI) and N1E-115 cell membranes was performed as described in the text. Bk and analogues were evaluated at 6 to 12 concentrations in triplicate and K_i (nM) values are the means of 2 to 3 independent determinations which varied less than 20%.

* Thi, β-(2-thienyl)-L-alanine; Hyp, L-4-hydroxyproline.

Small changes in Bk analogue structure had significant effects on agonist or antagonist effects on N1E-115 cell Bk receptor-mediated PI turnover, which differ from their contractile effects in smooth muscle. While [Thi^{5,8}, D-Phe^7]-Bk displayed antagonist activity and [D-Phe^7]-Bk showed weak agonist or antagonist activities in smooth muscles, they both exhibited agonist activity on N1E-115 cell PI turnover and failed to inhibit Bk stimulation at concentrations as high as 1 μM. Additional substitution of Hyp for Pro at position 3 of [Thi^{5,8}, D-Phe^7]-Bk changed the agonist to a pure antagonist. Amino terminal extended Bk analogues also competitively inhibited Bk-stimulated PI turnover. Removal of the carboxyl-terminal Arg from Bk in [des-Arg^9]-Bk abolished activity at both the receptor binding sites and bioassay.

Discussion Binding affinities and the agonist or antagonist activities of Bk structural analogues observed here suggest multiple Bk receptor binding sites. The inactivity of [des-Arg^9]-Bk indicates that the receptor effects we have examined reflect the B_2 subtype. Specific receptor recognition sites differed for the Bk analogues in guinea-pig ileum and N1E-115 neuroblastoma cell membranes. The relative agonist or antagonist actions varied among the Bk derivatives. [D-Phe^7]-Bk is a pure antagonist of Bk elicited guinea-pig ileum contractions, while it is a weak agonist in the rat uterus contraction assay (Vavrek & Stewart, 1985a; Stewart & Vavrek, 1986). [Thi^{5,8}, D-Phe^7]-Bk, however, acts as a pure antagonist of both rat uterus and guinea-pig ileum smooth muscle contractions. Strikingly, in this study, both [D-Phe^7]-Bk and [Thi^{5,8}, D-Phe^7]-Bk displayed pure agonist activity on PI turnover in N1E-115 neuroblastoma cells. The receptor coupled responses therefore, differ in the three systems examined.

The apparent physiological roles of Bk in pain, inflammation and blood pressure regulation, suggest a therapeutic role for the Bk antagonists. The differential effects of these Bk derivatives in several bioassays imply a multiplicity of Bk B_2-receptor subtypes which may have therapeutic relevance in the development of organ specific antagonists.

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