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Prolonged signalling and trafficking of the bradykinin B_2 receptor stimulated with the amphibian peptide maximakinin: insight into the endosomal inactivation of kinins

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Abstract

Maximakinin, a 19-residue peptide from the amphibian *Bombina maxima*, incorporates the full sequence of bradykinin (BK) at its C-terminus with a hydrophilic 10-residue N-terminal extension. As a putative venom component, it may stimulate BK B_2 receptors (B_2Rs) in a distinct manner relative to the fragile mammalian agonist BK. Maximakinin affinity for B_2Rs and angiotensin converting enzyme (ACE) and its pharmacological profile have been compared to those of BK. Maximakinin is an agonist of the human and rabbit B_2R with a 8-12 fold lesser potency, but a prolonged duration of action relative to BK (ERK MAP kinase activation, c-Fos induction in HEK 293 cells). Maximakinin had a moderately inferior affinity (~6-fold vs. BK) for recombinant ACE based on [^{3}H]enalaprilat binding displacement. Unlike BK, maximakinin induced the internalization of the fusion protein B_2R-green fluorescent protein (GFP) and the downregulation of this construction over a 12-h stimulation period, reproducing the effect of inactivation-resistant B_2R agonists. Alternate homologues of BK extended at the N-terminus showed intermediate behaviours between BK and maximakinin in the B_2R-GFP downregulation assay. The recycling of B_2R-GFP at the cell surface after a 3-h BK treatment was notably inhibited by cotreatment with E-64 or bafilomycin A1, supporting that an endosomal cysteine protease degrades kinins in a process that determines the cycling and fate of the B_2R. Maximakinin is the first known natural kinin sequence that elicits a prolonged cellular signalling, thus suggesting a possible basis for a venomous action and a naturally selected one for the design of B_2R-transported biotechnological cargoes.

Keywords : maximakinin, bradykinin B_2 receptors, receptor cycling, angiotensin converting enzyme, endosomes

1. Introduction

Maximakinin, a natural sequence isolated from the skin of the amphibian *Bombina maxima*, is composed of the full bradykinin (BK) sequence at its C-terminal region with a 10-residue N-terminal extension (a 19-mer; primary structure in Table 1); this peptide is reportedly pharmacologically active in mammalian tissues [1]. In the genome of *B. maxima*, there are 5 identical copies of the maximakinin coding sequence found in 2 predicted and cleavable proteins secreted from cells present in skin secretions [2], supporting that the peptide serves a vital function in this animal. Thus, if maximakinin is an ingredient of toxic and/or dissuasive animal venom, it may possess intrinsic signaling properties different from those of BK, e.g., exerting prolonged signaling at the high affinity B_2 receptor (B_2R). *Polistes* kinin is one of the BK-related peptides found in the venom of a subgroup of Hymenoptera insects; it resembles maximakinin by including the full BK sequence at its C-terminus and a hydrophilic and cationic N-terminal extension of 9 residues [3] (Table 1). Angiotensin converting enzyme (ACE), an ectopeptidase highly active to inactivate BK, exhibits a rapidly decreasing affinity for higher homologues of BK prolonged at the N-terminus, although this enzyme attacks the opposite end of the BK sequence by hydrolysing a C-terminal dipeptide; thus, *Polistes* kinin is completely protected from inactivation by ACE [4].

Recent discoveries based on the use of synthetic B_2R agonists show the role of endosomal inactivation in the duration of signalling and rate of recycling of endocytosed B_2R back to the cell surface. The agonist peptide B-9972 (D-Arg-[Hyp^3, Igl^5, Oic^7, Igl^8]-BK; Table 1) and a nonpeptide partial agonist drug structurally derived from an antagonist, compound 47a, integrate resistance to many or all peptidases that may inactivate BK (amino-, carboxy- and endopeptidases) and they provoked the endocytosis of B_2Rs for more than 12 h, with

consequences such as prolonged ERK1/2 and c-Fos signalling, prolonged B_2R association with β-arrestins in endosomes and measurable receptor downregulation (degradation) over 12 h [5, 6]. BK only induced short term signalling and fully reversible receptor internalization (in 1-3 h). Another laboratory has shown that endosomal degradation of the peptide substance P limits the β-arrestin-mediated signalling of the cognate NK_1 receptors [7].

We determined whether maximakinin had affinity for and stimulated mammalian B_2Rs and whether indications of its resistance to extra- or intracellular inactivation could be gathered in comparative experiments conducted with the mammalian agonist BK and some biologically active BK homologues presenting extended N-terminal sequences. Specifically, the duration of maximakinin-induced B_2R endocytosis and signalling and the possible long-term receptor downregulation in cells treated with this kinin were points of particular interest. Complementary experiments on the mechanism of endosomal inactivation of BK were conducted.

2. Methods

2.1. Radioligand binding competition assays

[^{3}H]BK (3 nM) was the radioligand exploited as described to determine the affinity of maximakinin by binding competition to recombinant B_2R-GFP receptors [6]. [^{3}H]Enalaprilat (1 nM) binding to recombinant human ACE (coded by the peACE vector, generous gift from Prof. P. Corvol, Paris, France) was determined in transiently transfected HEK 293a cells as described [8]; this binding is displaced by BK-related substrates [9], which was verified for maximakinin. BK was used in both binding assays as a reference.

2.2. Contractility assay

The institutional research ethics board approved the anonymous use of human umbilical cord segments obtained after elective cesarean sections. B_2 receptor agonists were assayed as described by the contraction of the human isolated umbilical vein [5, 6, 10]. Cumulative concentration-effect curves were constructed for maximakinin and its potency estimated by the half-maximal concentration (EC_{50}) and compared to that of the reference agonist BK.

2.3. Microscopy, cytofluorometry

Epifluorescence of GFP-tagged B_2 receptor (B_2R-GFP) was observed in HEK 293 cells that stably express this construction in order to detect maximakinin-induced receptor endocytosis and cycling as a function of time [5, 6]. The effects of this kinin were compared to those of BK and of the inactivation-resistant BK analogue, B-9972. A variant consisted of a B_2R-GFP recycling assay: the HEK 293 cells that stably express this fusion protein were stimulated with BK (100 nM) for a 3-h incubation period, after which the endocytosis of the receptor is fully reversible [5].

Some cells were co-treated with various enzyme inhibitors to analyze the mechanism of the recycling.

2.4. Immunoblots

HEK 293 cells stably expressing B_2R-GFP were treated with drugs for 30 min-12 h, lyzed and the immunoblots for B_2R-GFP were performed as previously reported [5], based on the monoclonal anti-GFP antibody JL8 (Clontech, Palo Alto, CA). The agonist action of maximakinin on the ERK1/2 MAP kinase phosphorylation assay was tested as described in HEK 293 cells stably expressing B_2R-GFP or in HEK 293a cells expressing myc-B_2Rs [5]. Induction of the expression of the transcription factor c-Fos is also a response to kinins in HEK 293a cells expressing myc-B_2Rs, and has been detected as described [5].

2.5. Drugs

BK was purchased from Bachem Biosciences (King of Prussia, PA), maximakinin and Met-Lys-BK, from Phoenix Pharmaceuticals (Burlingame, CA), Lys-BK and Ile-Ser-BK from Sigma-Aldrich. LF 16-0687 (anatibant; XY2405; 1-[[2,4-dichloro-3-[(2,4-dimethylquinolin-8-yl)oxy]methyl]phenyl]sulfonyl]-N-[3-[[4-(aminoiminomethyl]-phenyl]carbonylamino]propyl]-2(S)-pyrrolidinecarboxamide, mesylate salt), a previously described nonpeptide B_2R antagonist [11], was a gift from Laboratoires Fournier (Daix, France). B-9972 and carboxyfluorescein-ε-aminocaproyl-bradykinin (CF-εACA-BK) are peptide B_2R agonists (table 1); the former incorporates resistance to several peptidases [5] and the latter is a fluorescent probe characterized elsewhere [10]. Pepstatin A, phenylmethanesulfonyl fluoride (PMSF), N-(trans-epoxysuccinyl)-L-leucine 4-guanidinobutylamide (E-64) are respectively inhibitors of aspartyl, seryl and cysteinyl proteinases; these chemicals were purchased from Sigma-Aldrich. The ACE inhibitor

enalaprilat was from Kemprotec Ltd. (Maltby, Middlesbrough, United Kingdom), and the vacuolar ATPase inhibitor bafilomycin A1, from LC Laboratories (Woburn, MA, USA).

2.6. Data analysis

Results are presented as means ± SEM. Radioligand binding data were fitted by nonlinear regression to a one-site competition equation using a least-square method (Prism 4.0, GraphPad Software Inc., San Diego, CA) and IC_{50} values calculated from this procedure. The same computer program was used to draw concentration-effect curves (least square fitting of sigmoidal dose-response equation with variable slope) and to derive contractile EC_{50} values. ANOVA followed by Dunnett's test was used to compare sets of normally distributed numerical values to a common control; for nonnormal distributions, the Kruskal-Wallis test followed by Dunn's multiple comparison test were used for the same purposes (GraphPad Instat 3.0 program).

3. Results

Synthetic maximakinin exhibits a moderate loss of affinity relative to BK at the B_2 receptor (~12-fold in the competition assay for the radioligand binding to the rabbit receptor, ~8-fold in the human vein contractility; Fig. 1). The IC_{50} for the displacement of [^{3}H]BK binding to B_2R-GFP was 17.6 for BK (95% confidence interval 10.0-30.9 nM) and 216 nM (105-443 nM) for maximakinin; the contractile EC_{50} in the umbilical vein was 10 nM for BK (95% confidence interval 6.9-14.5 nM) and 83.2 nM for maximakinin (43.6-158.8 nM). BK displaced [^{3}H]enalaprilat binding to recombinant ACE; maximakinin was 6-fold less effective in this respect (Fig. 1C).

The HEK 293 cells that stably express B_2R-GFP exhibited, as an acute (30 min) response to BK and maximakinin, the accumulation of the phospho-ERK1/2 MAP kinases and induction of c-Fos expression (Fig. 2). On a molar basis, maximakinin was generally less potent than BK in these assays. These responses were abated in cells pretreated with the B_2R nonpeptide antagonist LF 16-0687. However, the BK-induced responses subsided subsequently in cells incubated for longer periods, which is not the case for the inactivation-resistant agonist B-9972 [5, 6]. The same holds true for c-Fos expression and ERK1/2 phosphorylation induced with maximakinin (response to BK present at 3 h, not 12 h; response to B-9972 and maximakinin recorded at both times; Fig. 3). Maximakinin is the first known natural sequence to behave as a persistent B_2R agonist, producing after 12 h of treatment as much phospho-ERK1/2 and c-Fos accumulation as that produced with B-9972 in B_2R-GFP expressing cells. Also, maximakinin produced B_2R-GFP downregulation as intensely as B-9972, with low molecular weight fragments appearing in both cases, down to free GFP (Fig. 3). BK was inactive at time 12 h when used at an equieffective concentration, as reported previously [6]. The two peptides that caused B_2R-GFP downregulation

induced a persistent endocytosis of this fusion protein (epifluorescence microscopy, Fig. 4), unlike BK that caused only an acute (30 min), but entirely reversible (3 h) translocation of the fluorescent receptor.

The resistance of maximakinin to endosomal inactivation possibly points out to the N-terminus of BK as structural determinant of vulnerability in this milieu. The B_2R-GFP downregulation assay was applied to other BK homologues (Table 1) in order to detect effects of alternate N-terminal prolongations (Fig. 5). For this series of experiments, HEK 293 cells stably expressing B_2R-GFP were treated for 12 h with a BK homologue at 1 μM, except for the low affinity agonist CF-εACA-BK, used at 5 μM [10]. Natural sequences derived from mammalian kininogens, Lys-BK, Met-Lys-BK and Ile-Ser-BK, were also tested as alternate N-terminally extended BK homologues. The 3 latter peptides did not significantly downregulate B_2R-GFP, as assessed by the decreases abundance of the fusion protein and simultaneous appearance of free GFP in total cell extracts, but Lys-BK and Met-Lys-BK exhibited a nonsignificant trend for a production of free GFP larger than control in this assay (immunoblots, Fig. 5). Further, the fluorescent B_2R agonist CF-εACA-BK downregulated the receptor almost as effectively as maximakinin, supporting that alternate N-terminal extensions provide protection against endosomal inactivation.

If the structure of the agonist determines the duration of the B_2R cycling by affecting the degradation of the ligand, it should be possible to exploit the recycling of B_2R-GFP to the plasma membrane in BK-stimulated cells (as in Fig. 4) to identify the pathways of BK degradation by co-treating the intact cells for 3 h with enzymatic inhibitors. This “recycling inhibition assay” (Fig. 6) revealed that the cysteine protease inhibitor, E-64, blocked B_2R-GFP recycling to the cell

surface, unlike inhibitors of aspartyl or seryl proteases, pepstatin A or PMSF, respectively (Fig. 6). Additional inhibitors that showed some activity were the ACE blocker enalaprilat (partial recycling inhibition) and bafilomycin A1, a substance that inhibits the proton pump that acidifies intracellular organelles (Fig. 6).

4. Discussion

4.1. Effect of N-terminal structure of kinins on affinity for the B_2R

Maximakinin has direct affinity for the mammalian B_2R in a binding assay conducted on ice in the presence of peptidase and protease inhibitors (Fig. 1A), suggesting that it is not a precursor transformed by the metabolism into BK or some other homologue of BK. The slight loss of affinity for the B_2R is determined by the N-terminal extension in maximakinin, one log unit on the average. In the current model of BK docking to the B_2R, a G protein coupled receptor, the C-terminal part of the ligand interacts with the trans-membrane domains, while the N-terminal end of BK rather binds to an extracellular loop [12]. Thus, N-terminally extended BK sequences may retain receptor affinity despite the limited accessibility of the B_2R extracellular surface determined by heavy glycosylation [13]. In support of this, Met-Lys-BK exhibits an affinity only marginally inferior to that of BK for the recombinant human and mouse B_2Rs [14] and Ile-Ser-BK, a sequence derived from rodent T-kininogen, also retains a good fraction (10-50%) of BK potency at mammalian B_2Rs [15]. On the other hand, we recently reported an N-terminal extended analog, CF-ε-ACA-BK, that was 400-1000-fold less potent than BK as an agonist of the B_2R [10] (Table 1). Present results suggest that considerable N-terminal extensions may be tolerated in B_2R agonists if the amino acid sequence is optimized. In that regard, maximakinin exhibits an amphibian peptide extension that is hydrophilic and possesses a possible “hinge” spatially oriented by Gly^{-1}-Pro^{0}, perhaps allowing more flexibility to meet binding requirements *Polistes* kinin, another higher homologue of BK from an animal venom, has a glycine residue at the -1 position (table 1), which may be structurally equivalent [3].

4.2. Extracellular inactivation of maximakinin

In the HEK 293/B_2R-GFP cellular system, soluble ACE present in the fetal bovine serum-containing culture medium mediates the main extracellular inactivation pathway for BK ($t_{1/2} < 10$ min) [16]. HEK 293 cells do not express ACE unless transfected with a corresponding expression vector, based on both a [^{3}H]enalaprilat binding assay and an enzymatic assay [8, 17]. The partial inhibition of B_2R-GFP recycling in 3 h in the presence of enalaprilat (Fig. 6) may be interpreted as the effect of the ~13-fold prolongation of the BK half-life in the culture medium when extracellular ACE is blocked [16]. However, ACE blockade does not importantly inhibit B_2R-GFP recycling over a longer period (12 h) and leads only to a minor breakdown of the B_2R-GFP protein relative to that induced by B-9972 [5]. Maximakinin is less active than BK to displace [^{3}H]enalaprilat from ACE, an indication of a moderately lower affinity for the former peptide, but resistance to ACE does not explain the long-term effects of maximakinin on receptor signalling, cycling and downregulation.

4.3. Intracellular inactivation of maximakinin and effects on signalling and B_2R downregulation

Additional and ill-characterized endosomal peptidases degrade BK after the ligand-B_2R complex internalization [18]. In endosomes, there is evidence that the ligand-B_2R-β-arrestin complex is stable, based on the colocalization of CF-εACA-BK and β-arrestin [10] or of B_2R-GFP with arrestins [6], and that signalling originates from such intracellular complexes [19]. Maximakinin stands out as an agonist resistant to intracellular inactivation based on the kinetics of both the signalling (Fig. 3) and B_2R-GFP cycling (Fig. 4). Thus maximakinin may be considered as a biased agonist of the BK B_2R, not because it induces a receptor conformation distinct from that induced by BK, but because differential receptor cycling will favour some signalling pathways (e.g., c-Fos) at the expense of others. Maximakinin is a natural amino acid sequence that points out to the N-terminus as the rate-limiting structural determinant of endosomal inactivation for

BK, the only difference between the 2 peptides being in this region. This conjecture is confirmed by the striking effect of the N-terminally prolonged fluorescent BK analogue on B_2R-GFP abundance and by the intermediary behaviour of other homolog peptides such as Lys-BK, when assessed for this outcome (Fig. 5). Vacuolar ATPase is the proton pump that acidifies ensodomes, lysosomes and other organelles [20]; its inhibitor bafilomycin A1 reduced the recycling of B_2R-GFP in cells stimulated with BK for 3 h (Fig. 6), supporting that the BK-inactivating enzyme(s) operate at an acidic pH. Candidate endosomal peptidases that inactivate BK do not include endothelin-converting enzyme-1, while the latter is important for the cycling of substance P, calcitonin gene-related peptide and their respective receptors [7, 21]. Endosomal/lysosomal cathepsins include cysteinyl, seryl and aspartyl proteases [22], and the effect of inhibitors of these enzyme classes on B_2R-GFP recycling points out to the role of cysteinyl proteinase(s) in the inactivation of BK (Fig. 6). Cathepsin K has been previously shown to degrade BK, but has a very limited distribution (mostly osteoclasts) [23]. BK is reportedly not inactivated by cathepsins B, C, L and S [23-25], while conflicting evidence has been produced for cathepsin H [23, 26]. More work is needed to ascertain the identity of this endosomal kininase.

4.4. Biological and clinical implications

B_2Rs are widely distributed in the gastrointestinal tract, including in enterocytes, neural plexus and smooth muscle cells [27, 28], and may mediate the dissuasive effects of a persistent agonist such as maximakinin in a hypothetical predatory species in the wildlife. The toxicity of venom polycationic peptides may exceed the stimulation of the B_2Rs, as *Polistes* kinin is a direct histamine releaser from mast cells, BK being ineffective in this respect [29].

Large cargoes can be transported by the activated BK B_2R, for instance antibody-based cargoes in excess of 2 MDa [30]. The particular issue with the BK B_2R is the unpredictable affinity tolerance of extended agonist peptides. The extended sequence showcased by maximakinin may supply a naturally selected solution to this problem**.** For instance, a fluorescent B_2R probe of higher affinity than CF-ε-ACA-BK could be based on maximakinin and applied to cytofluorometric diagnostic. The long intracellular persistence of the peptide makes it ideal for *in vivo* B_2R mapping, for instance if labelled with an isotope adapted to positron emission tomography; the agonist version was recently found superior to an antagonist ligand in a study of another receptor type [31].

4.5. Conclusions

Maximakinin is the first known natural kinin sequence that elicits a prolonged cellular signalling, thus hitting at a possible basis for a venomous action. The N-terminal structure of kinins seems to determine susceptibility to the degradation process mediated by endosomal cysteinyl protease(s) that ultimately control the cycling and fate of the B_2R. Thus, maximakinin induces a prolonged endocytosis of B_2R that favors downregulation over receptor recycling to the cell surface. The fair affinity of this peptide for the B_2R and its intracellular persistence may support the future design of cargo-conjugated ligands.

Acknowledgements

This work was supported by the grant MOP-93773 from the Canadian Institutes of Health Research (CIHR) and by a Studentship Award to M.-T. B. from the Fonds de la Recherche en Santé du Québec. We thank Dr. Marc Pouliot (CHUQ) for facilitating the access to microscopic equipment and Ms. Johanne Bouthillier for technical assistance.

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Figure legends

Fig. 1. Pharmacology of maximakinin. A. Competition of [^{3}H]bradykinin (3 nM) binding to HEK 293 cells stably expressing B_2R-GFP by bradykinin (BK) and maximakinin. Values are the means ± S.E.M. of the number of duplicate determinations indicated by *n*. B. Agonist effect of BK and maximakinin on the human umbilical vein contractility assay. The maximal effect of maximakinin mediated by the endogenous B_2 receptors has been estimated by exposing tissues to a maximal concentration of BK. Values are means ± S.E.M. of the number of replicates indicated by *n*. C. Competition of [^{3}H]enalaprilat (1 nM) binding to HEK 293a cells transiently expressing human ACE by BK homologs. Values are the means ± S.E.M. of the number of duplicate determinations indicated by *n*.

Fig. 2. Acute signaling effect of BK and maximakinin in HEK 293 cells that stably express B_2R-GFP. Immunoblots for phospho-ERK1/2 and for c-Fos are shown as a function of stimulation with BK or maximakinin (concentrations as indicated, 30 min), optionally combined with the B_2R antagonist LF 16-0687 (1 µM, applied 15 min before agonists). Total ERK1/2 and β-actin were also immunoblotted to document equal track loading. Representative results of two separate experiments.

Fig. 3. Chronic effect of agonists on B_2R-GFP expressed in HEK 293 cells: immunoblot of total cell extracts based on anti-GFP antibodies (for the detection of the intact fusion protein and of GFP-containing fragments) and on antibodies relevant for prolonged signaling (presentation as in Fig. 2). The cells were submitted to the indicated treatments for 12 h in the regular culture

medium containing heat-inactivated FBS before extraction. The disappearance of the ~101 kDa band corresponding to the fusion protein and parallel increased of free GFP is interpreted as agonist-induced downregulation.

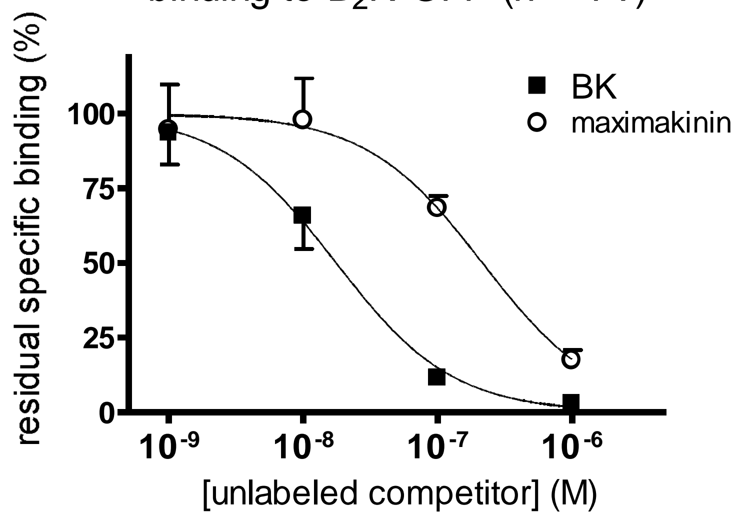
Fig. 4. Epifluorescence microscopy studies of live HEK 293 cells stably expressing B_2R-GFP and stimulated for 30 min, 3 h or 12 h with B_2R ligands at the indicated concentrations. Control cells generally exhibit sharply defined plasma membrane-associated green fluorescence. Blue fluorescence: nuclear counterstain with Hoechst 33258. Original magnification 1000 ×.

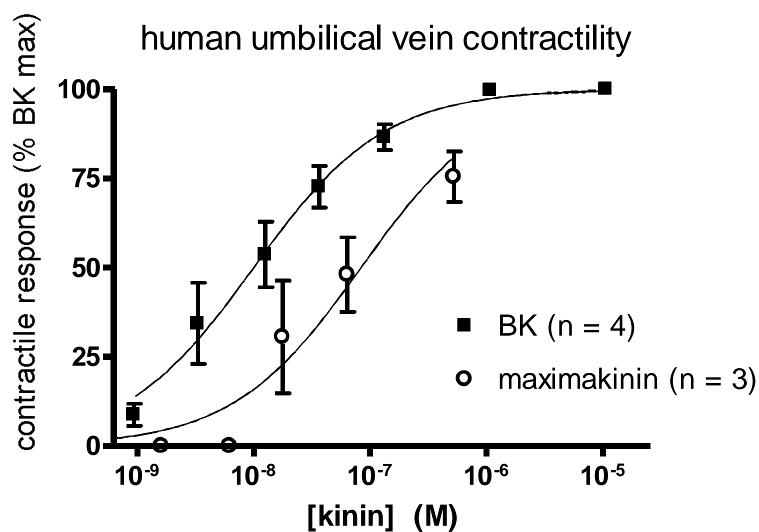
Fig. 5. B_2R-GFP down-regulation assay applied to BK homologues (12-h stimulation of HEK 293a cells with 1 µM of the indicated peptide, except for CF-εACA-BK used at 5 µM, followed by total cell extraction and immunoblot for GFP as in Fig. 4). Bottom: densitometry applied to repeated experiments. ANOVA determined that values corresponding to the substrate B_2R-GFP and those to the product GFP were heterogeneous ($P<10^{-4}$ and $<10^{-3}$, respectively). Dunnett's test was used to compare the effect of each treatment to control values (* $P<0.05$; ** $P<0.01$).

Fig. 6. B_2R-GFP recycling assay. Epifluorescence microscopy studies of live HEK 293 cells stably expressing B_2R-GFP, stimulated for 3 h with BK (100 nM) and co-treated as indicated. Original magnification of the green fluorescence 1000 ×. Three pictures from each experimental condition are shown.

Figure 1

Fig. 1





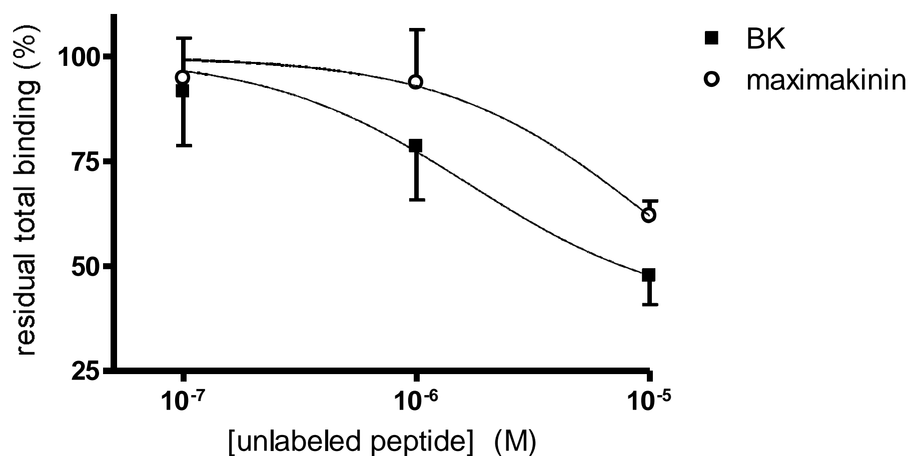


Figure 2

Fig. 2

+ LF 16-0687 1 μM

control
BK 100 nM
maximakinin 100 nM
maximakinin 1 μM
control
BK 100 nM
maximakinin 100 nM
maximakinin 1 μM

p-ERK1/2

total ERK1/2

c-Fos

β-actin

Figure 3
Fig. 3

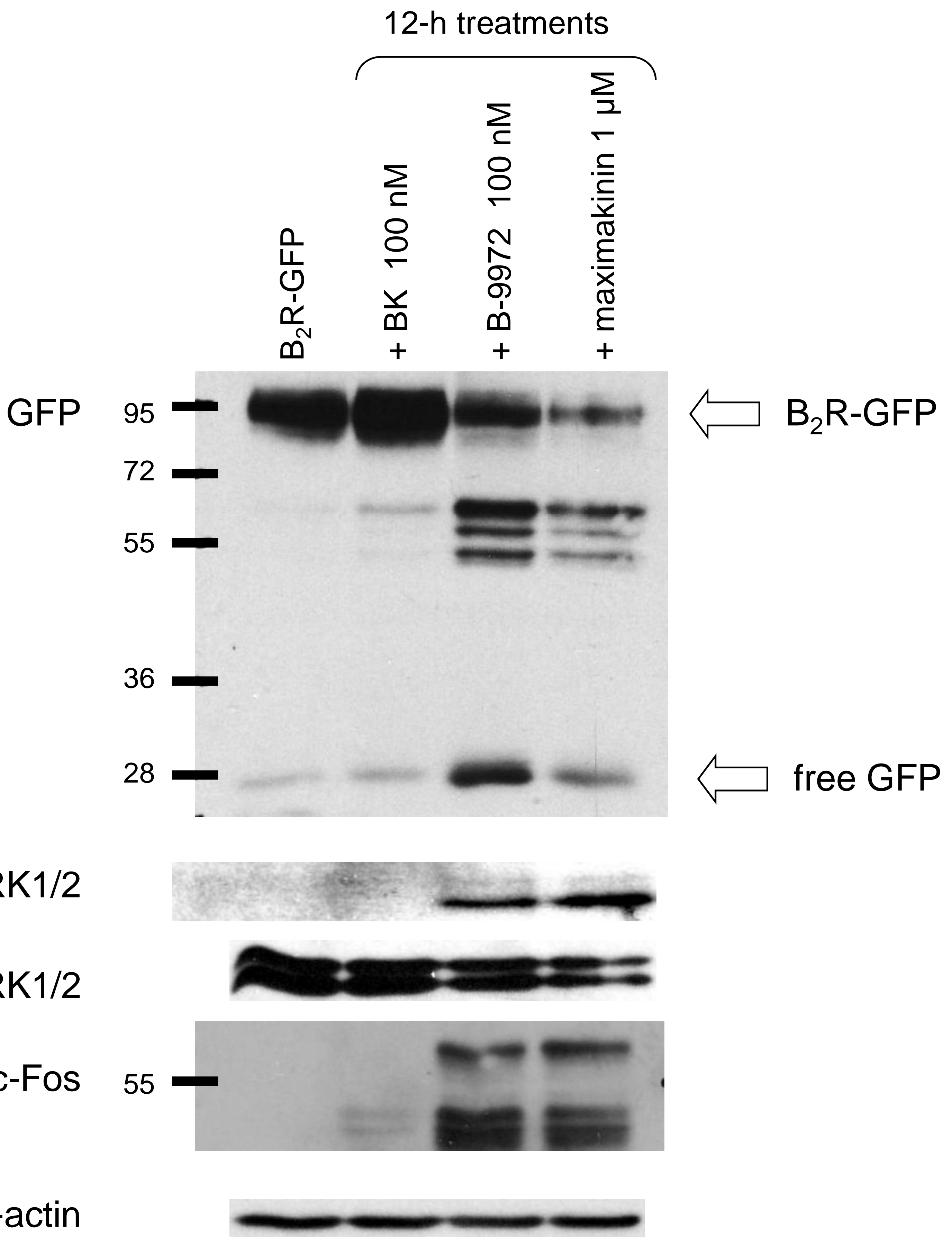
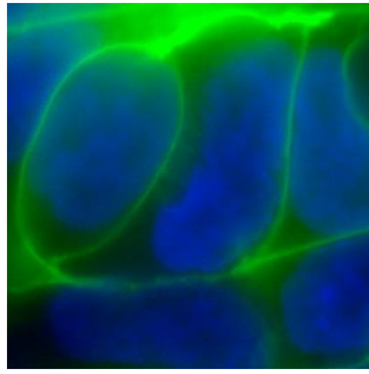
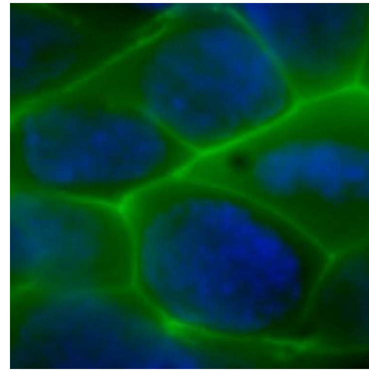


Figure 4

Fig. 4

controls



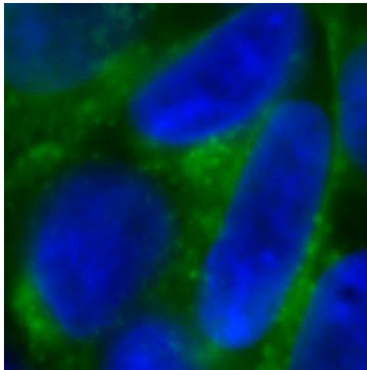


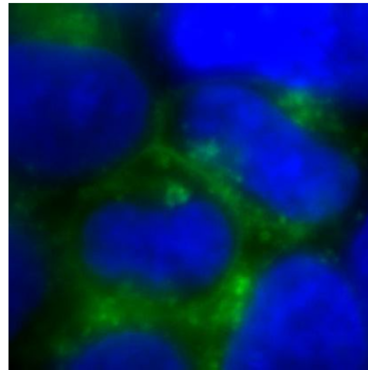
BK 100 nM

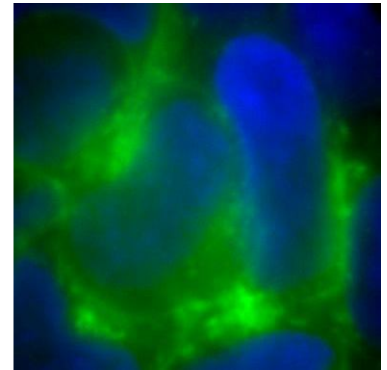
B-9972 100 nM

maximakinin 1 µM

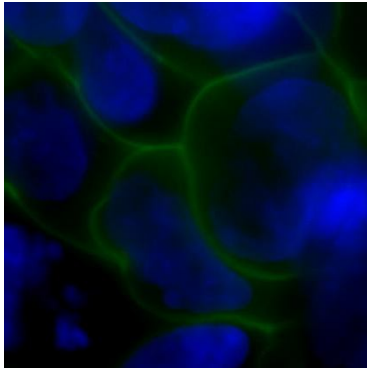
30 min

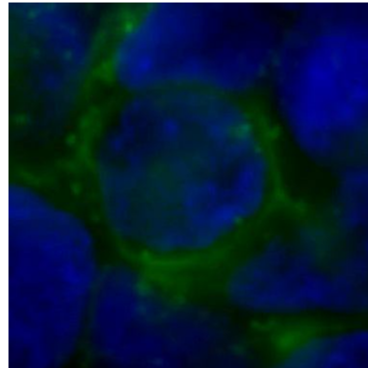


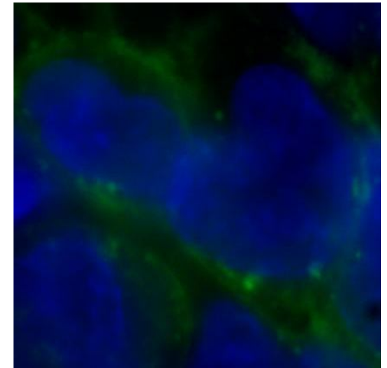




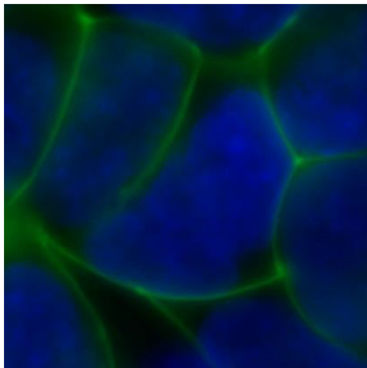
3 h

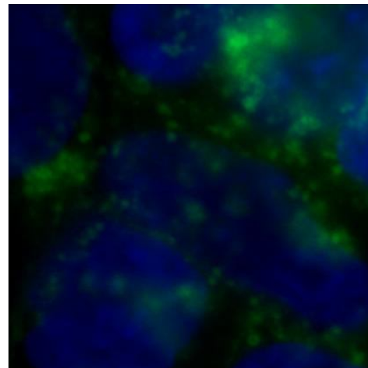






12 h





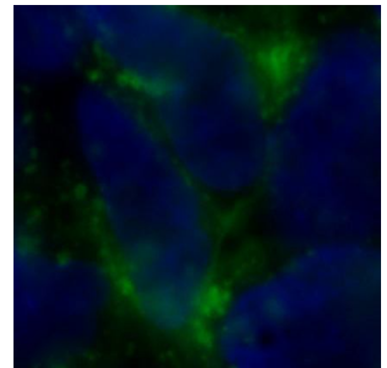


Figure 5

Fig. 5

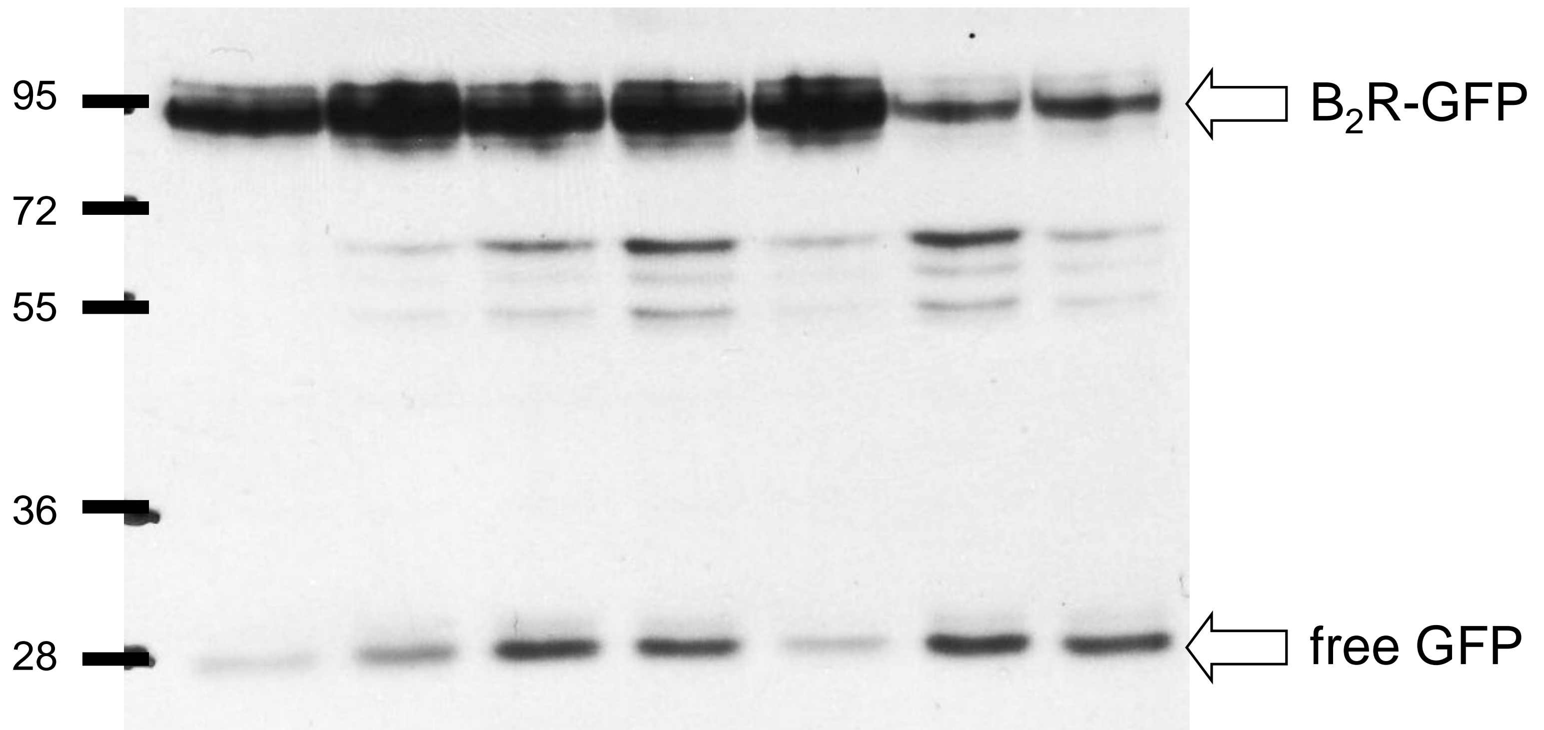


Figure 6

Fig. 6

recycling of B_2R-GFP following BK 100 nM, 3 h

co-treatment:

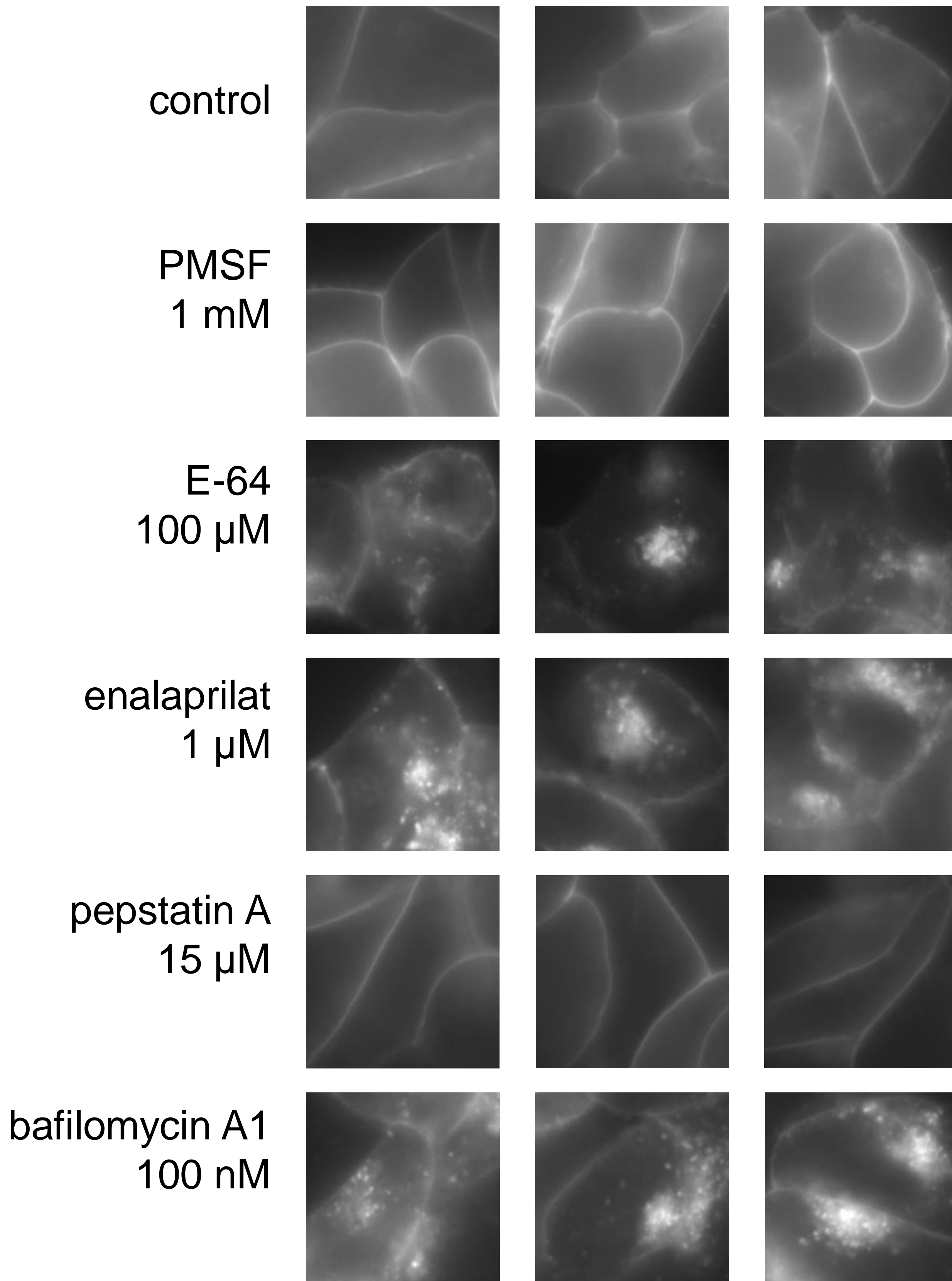


Table 1

Table 1. Primary structure of bradykinin (BK)-related peptides exploited or discussed in the present work.[a]

agonist peptide	position																		
	-9	-8	-7	-6	-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9
BK											Arg	Pro	Pro	Gly	Phe	Ser	Pro	Phe	Arg
Lys-BK										Lys	Arg	Pro	Pro	Gly	Phe	Ser	Pro	Phe	Arg
Met-Lys-BK									Met	Lys	Arg	Pro	Pro	Gly	Phe	Ser	Pro	Phe	Arg
Ile-Ser-BK									Ile	Ser	Arg	Pro	Pro	Gly	Phe	Ser	Pro	Phe	Arg
CF-εACA-BK									CF	εACA	Arg	Pro	Pro	Gly	Phe	Ser	Pro	Phe	Arg
maximakinin	Asp	Leu	Pro	Lys	Ile	Asn	Arg	Lys	Gly	Pro	Arg	Pro	Pro	Gly	Phe	Ser	Pro	Phe	Arg
***Polistes* kinin**	pyro-Glu		Thr	Asn	Lys	Lys	Lys	Leu	Arg	Gly	Arg	Pro	Pro	Gly	Phe	Ser	Pro	Phe	Arg
B-9972										D-Arg	Arg	Pro	Hyp	Gly	Igl	Ser	Oic	Igl	Arg

[a] Unconventional abbreviations : εACA : ε-aminocaproyl; CF : 5(6)-carboxyfluorescein ; Hyp: *trans*-4-hydroxyprolyl; Igl: α-(2-indanyl)glycyl; Oic: (3as, 7as)-octahydroindol-2-yl-carbonyl.

*Graphical Abstract