The 8th International Electronic Conference on Medicinal Chemistry (ECMC 2022)

01–30 NOVEMBER 2022 | ONLINE

Prodrugs activated by vascular ectopeptidases: proof of concept

Chaired by DR. ALFREDO BERZAL-HERRANZ;
Co-Chaired by PROF. DR. MARIA EMÍLIA SOUSA

pharmaceuticals

MDPI

François Marceau [1,*]

[1] Honorary Professor, Professor (retired), Department of Microbiology, Infectious Disease and Immunology, Université Laval, Québec, QC, Canada

* Corresponding author: francois.marceau@crchudequebec.ulaval.ca

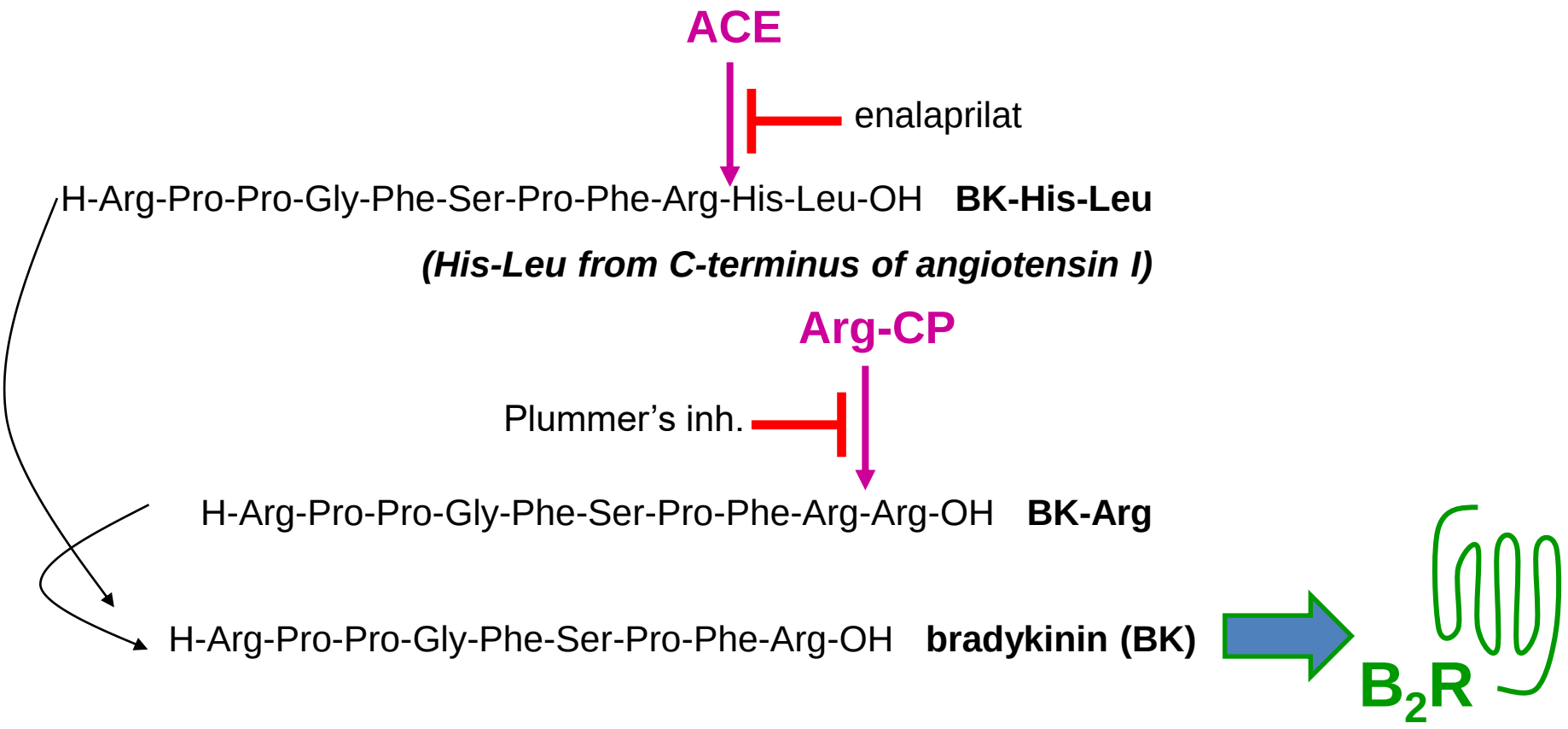
UNIVERSITÉ LAVAL

CHU de Québec Centre de recherche

Maladies Infectieuses & Immunitaires

Prodrugs activated by vascular ectopeptidases: proof of concept

Graphical Abstract



Abstract: Several vascular ectopeptidases reside in blood vessels and efficiently regulate peptide hormones. For instance, bradykinin (BK) is inactivated by angiotensin converting enzyme (ACE) or arginine-carboxypeptidases (Arg-CPs) and aminopeptidase N (APN) cleaves several substrates. Whether such peptidases may activate latent prodrugs of vasoactive agents has been tested. The contractility of the isolated human umbilical vein, radioligand binding assays, immunolocalization of peptidases, the internalization cycle of fluorescent receptors (microscopy), blood pressure measurements in anesthetized rats and specific inhibitors of peptidases have been exploited to show the feasibility of prodrug activation by vascular peptidases. L-Alanyl-histamine has virtually no affinity for the human histamine H1 receptor, but releases histamine and contracts the vein following cleavage by endogenous APN. Prolonged sequences of BK, such as BK-Arg, Arg^0-BK-Arg-Arg and BK-His-Leu have little affinity for the BK B2 receptor but are contractile in the umbilical vein and hypotensive in anesthetized rats via their action on this receptor type following cleavage of the C-terminal extensions by Arg-CPs for the first two peptides, also by ACE for the last two. Vascular ectopeptidases were shown to activate latent agonists of the H1 and B2 receptors, a concept that could be extended to various classes of drugs for a local action on the vasculature.

ECMC 2022
The 8th International Electronic Conference on Medicinal Chemistry
01–30 NOVEMBER 2022 | ONLINE

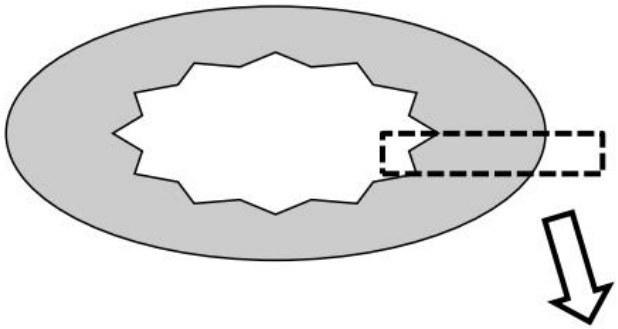
Introduction

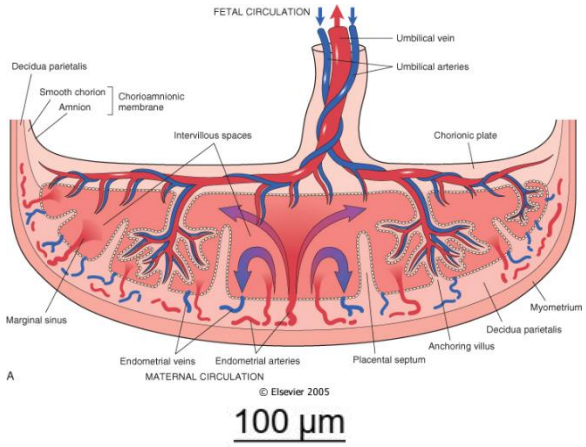
Peptidases expressed in vascular tissue are important modulators of the pharmacology of vasoactive peptides.

A “prodrug” strategy where a vasoactive agonist is activated only at the level of the microcirculation by resident peptidases is presented.

Such prodrug, containing one or more cleavable amino acids, would ideally massively lose affinity for the receptor of the active drug, but would be regenerated by the selected amino- or carboxypeptidase.

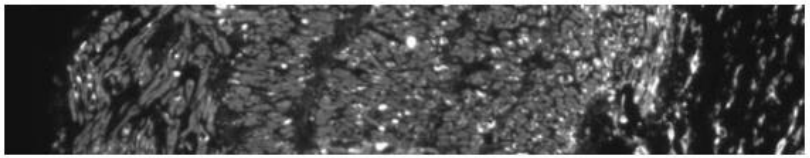
human umbilical vein



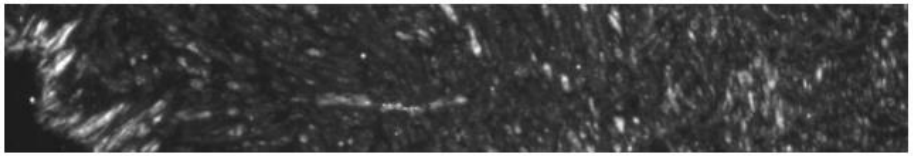


100 µm

α-actin monoclonal

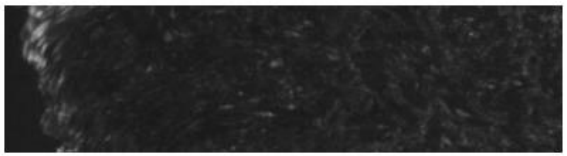


APN (CD13)

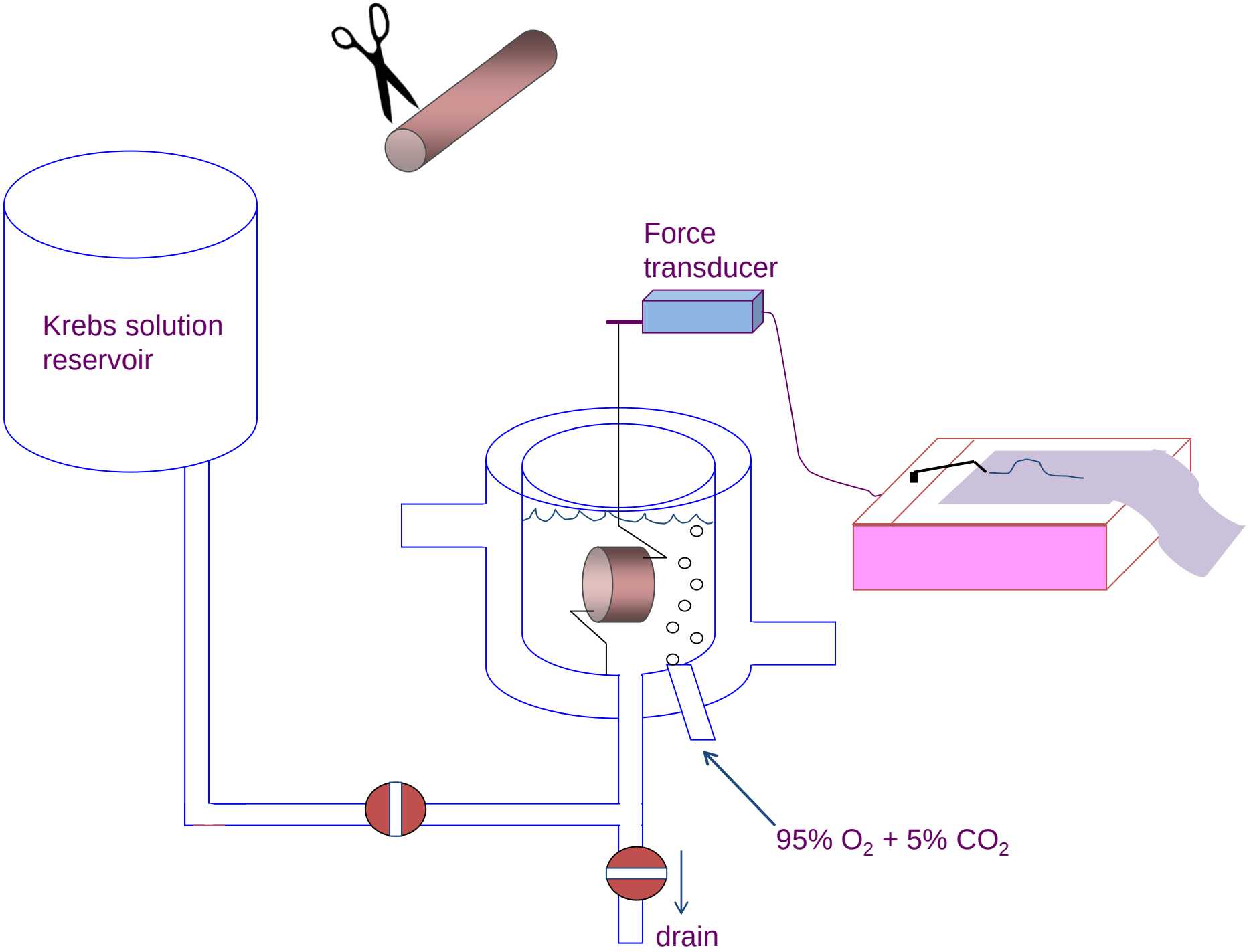


2nd anti-mouse Ab

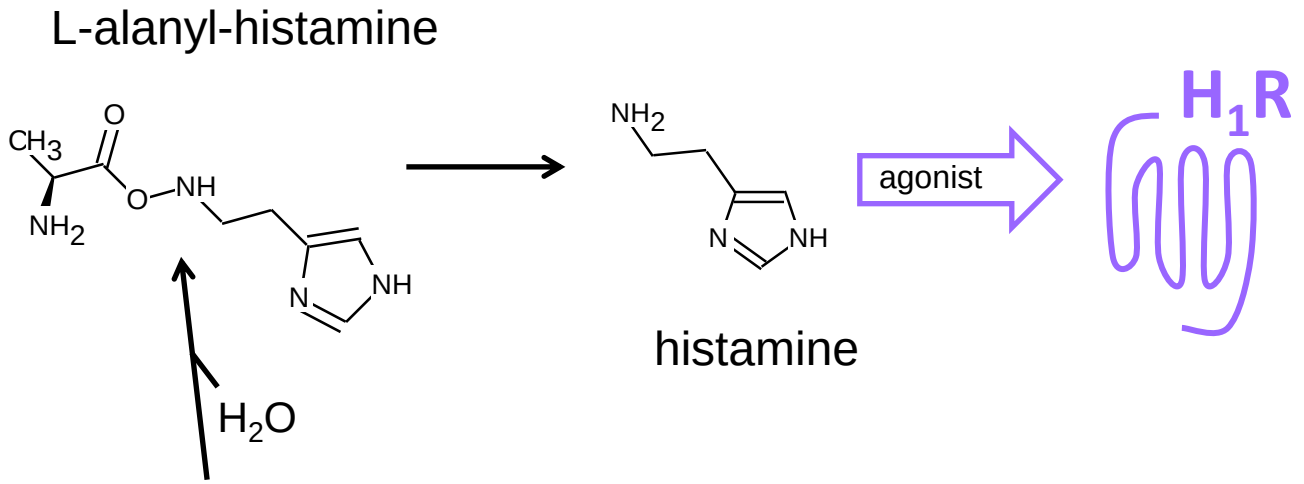
ACE polyclonal

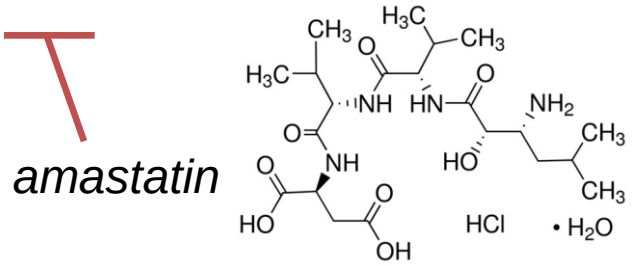


2nd anti-rabbit Ab

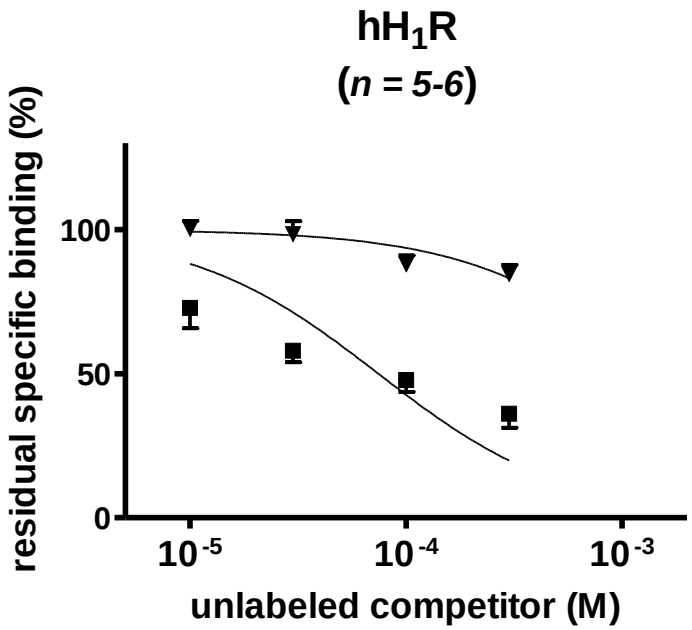

Krebs solution reservoir
Force transducer
95% O_2 + 5% CO_2
drain

1. A primary amine released by vascular aminopeptidase N

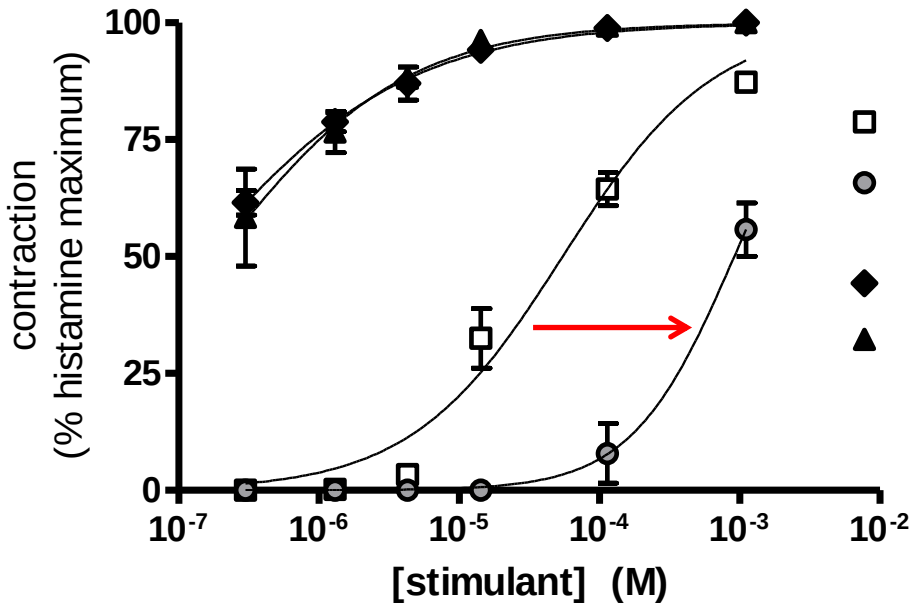




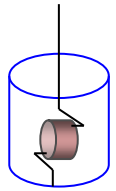
Int. Immunopharmacol. 2013; 17: 677



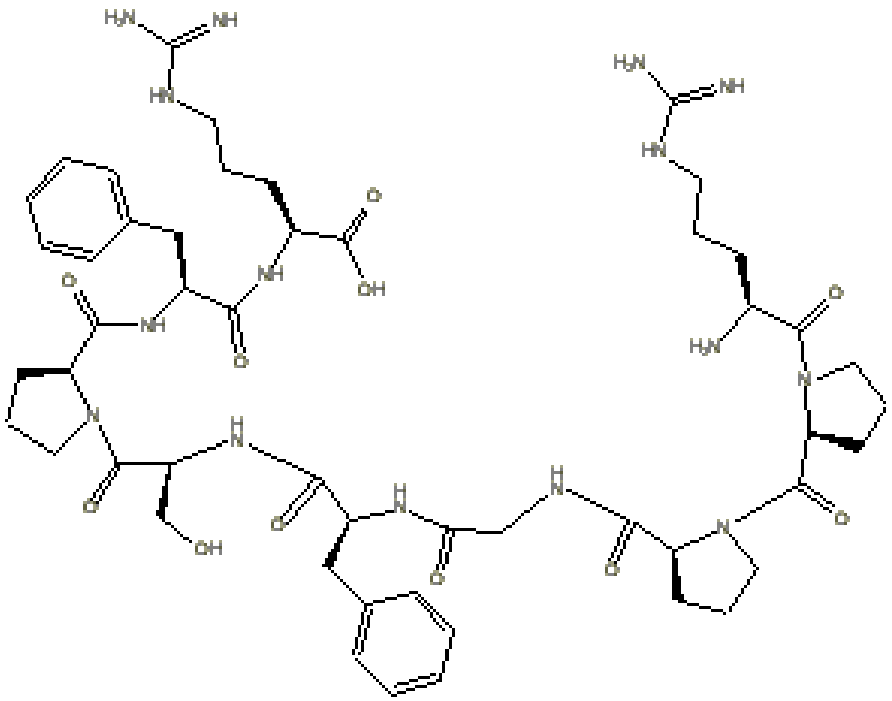
Binding competition of [^{3}H]pyrilamine (2 nM) to human recombinant H_1R

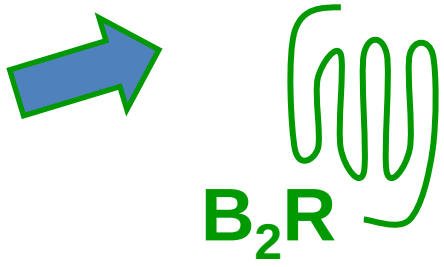


Umbilical vein contractility

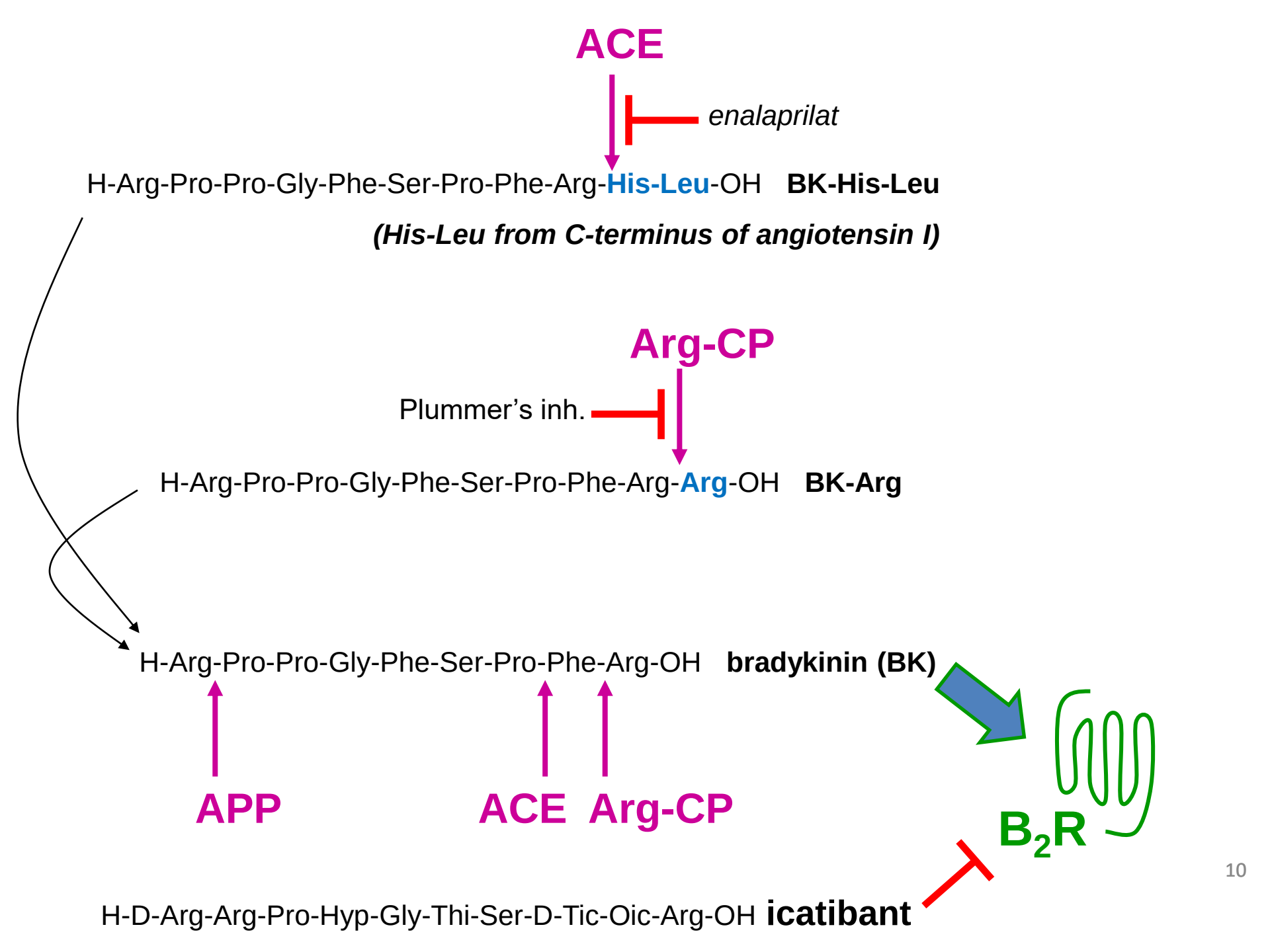


2. Extended bradykinin sequences activated by arginine-carboxypeptidases (kininase I) and angiotensin converting enzyme (kininase II)

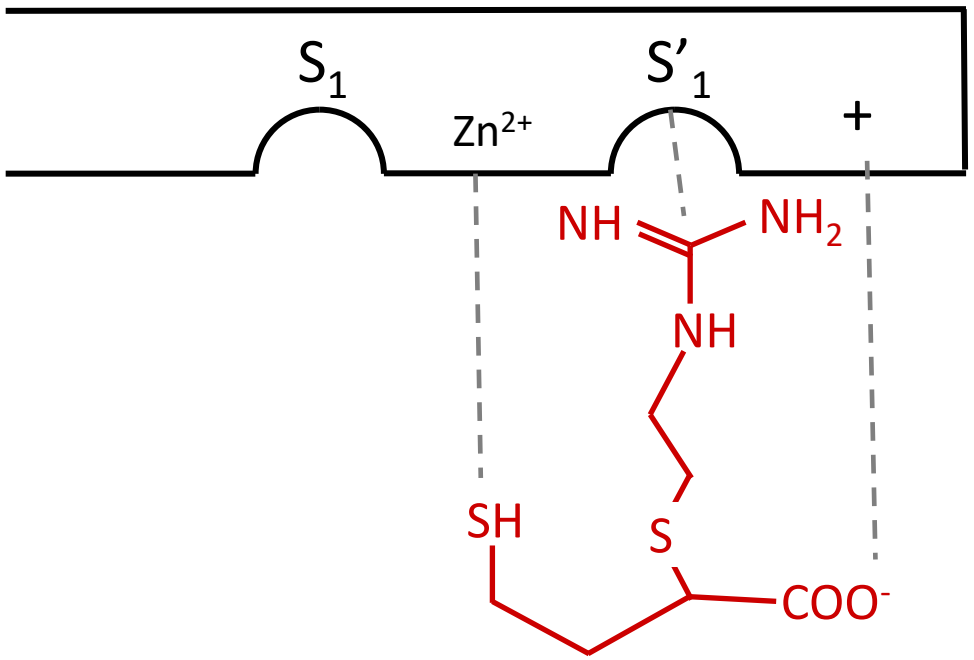




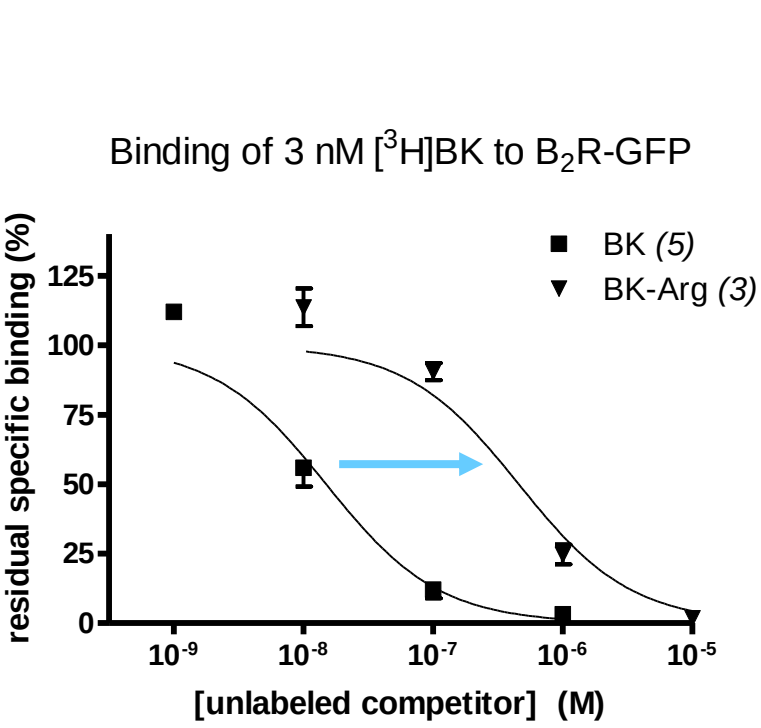
Front. Pharmacol.
2014; 5: 32
2016; 6: 306
2018; 9: 273

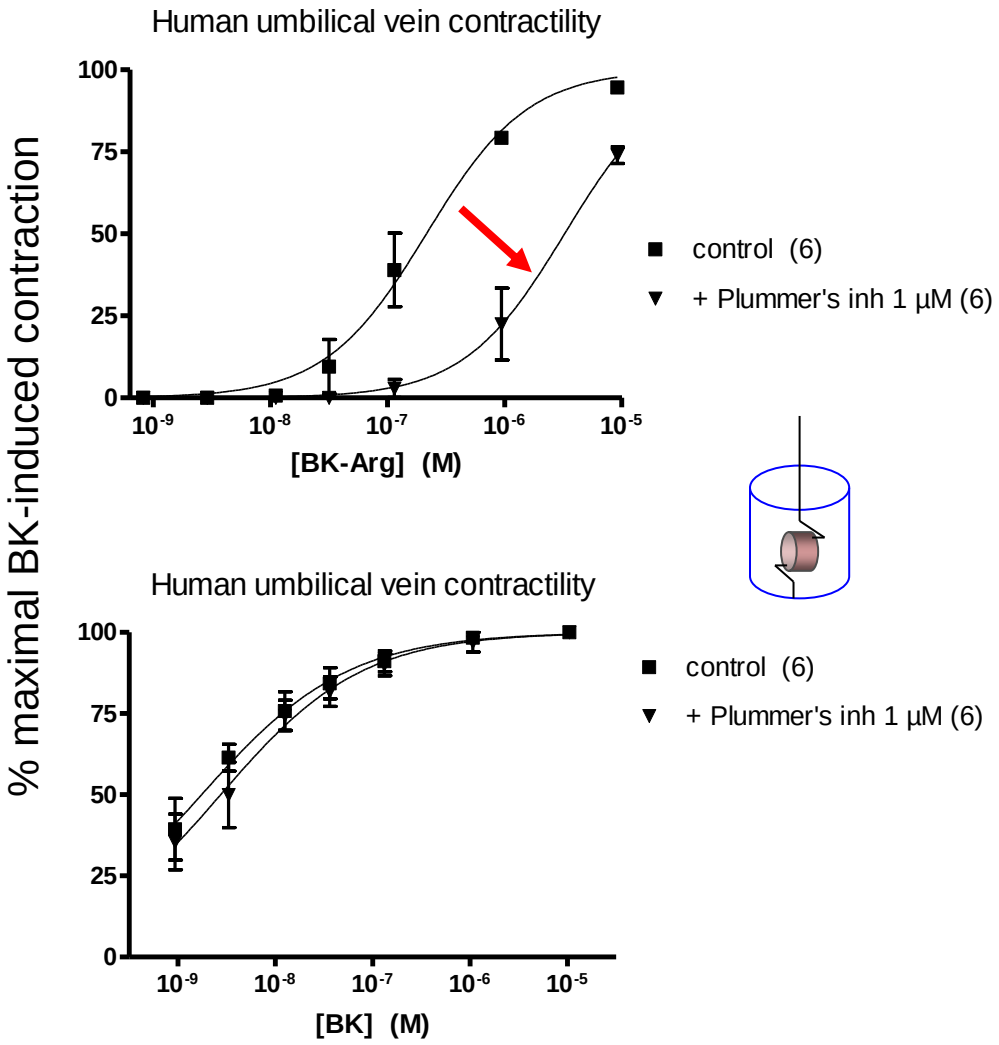

ACE
enalaprilat
H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-His-Leu-OH BK-His-Leu
(His-Leu from C-terminus of angiotensin I)
Arg-CP
Plummer's inh.
H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-Arg-OH BK-Arg
H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH bradykinin (BK)
APP
ACE
Arg-CP
B2R
H-D-Arg-Arg-Pro-Hyp-Gly-Thi-Ser-D-Tic-Oic-Arg-OH icatibant

Arg-CP

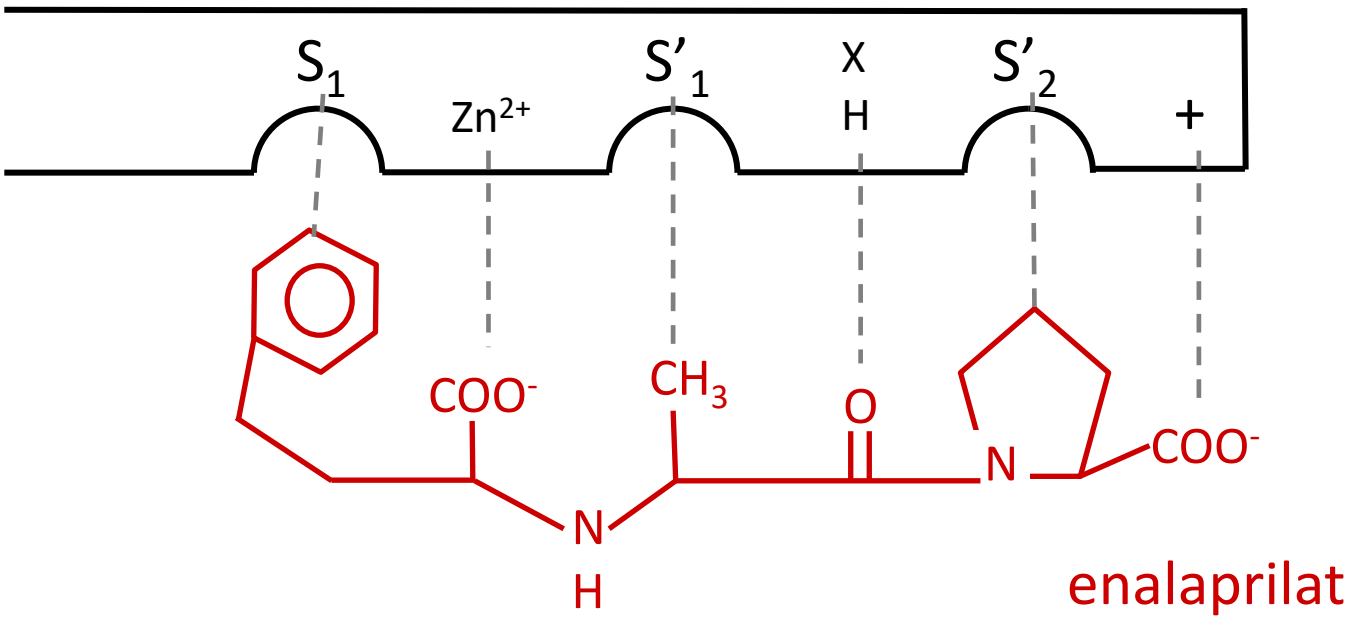


Plummer's inh

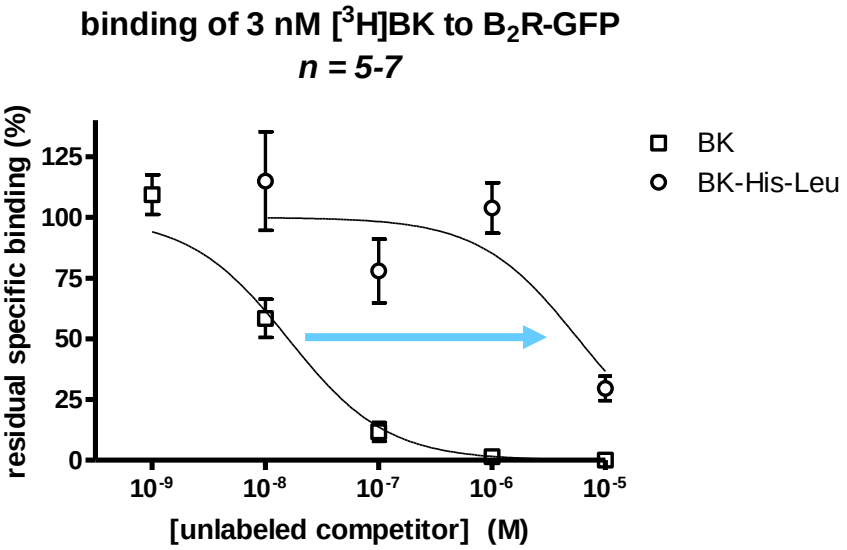

Binding of 3 nM [3H]BK to B2R-GFP
BK (5)
BK-Arg (3)
residual specific binding (%)
125
100
75
50
25
0
10-9
10-8
10-7
10-6
10-5
[unlabeled competitor] (M)

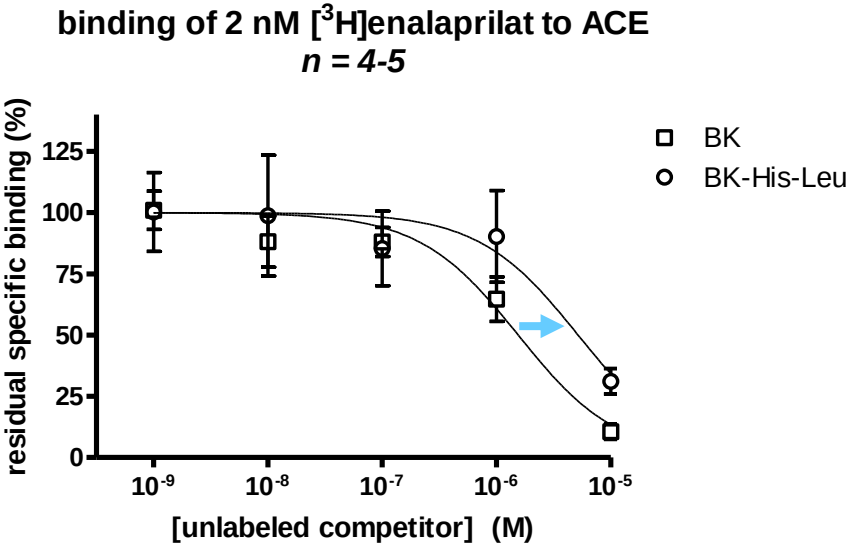

Human umbilical vein contractility
% maximal BK-induced contraction
100
75
50
25
0
10-9
10-8
10-7
10-6
10-5
[BK-Arg] (M)
control (6)
+ Plummer's inh 1 µM (6)
Human umbilical vein contractility
[BK] (M)
control (6)
+ Plummer's inh 1 µM (6)

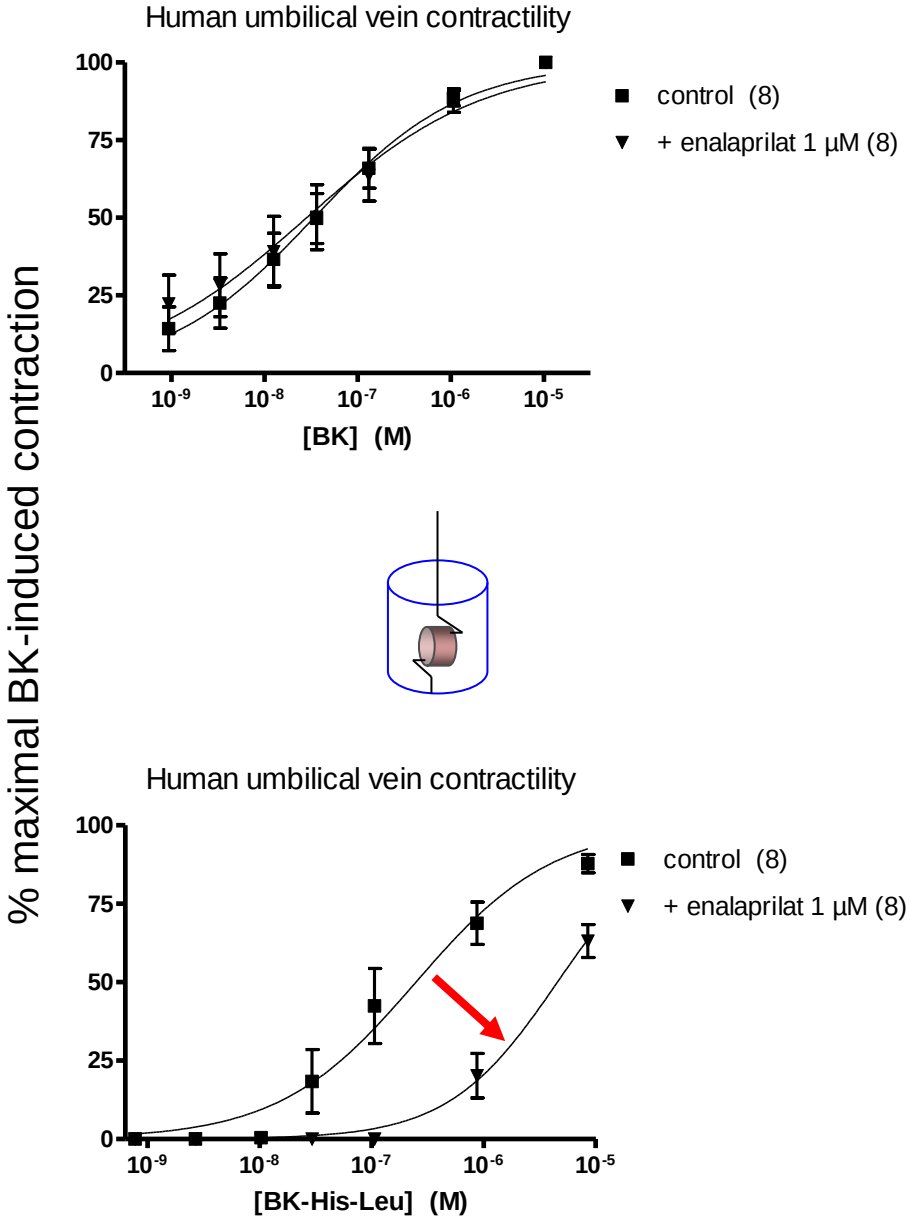
ACE

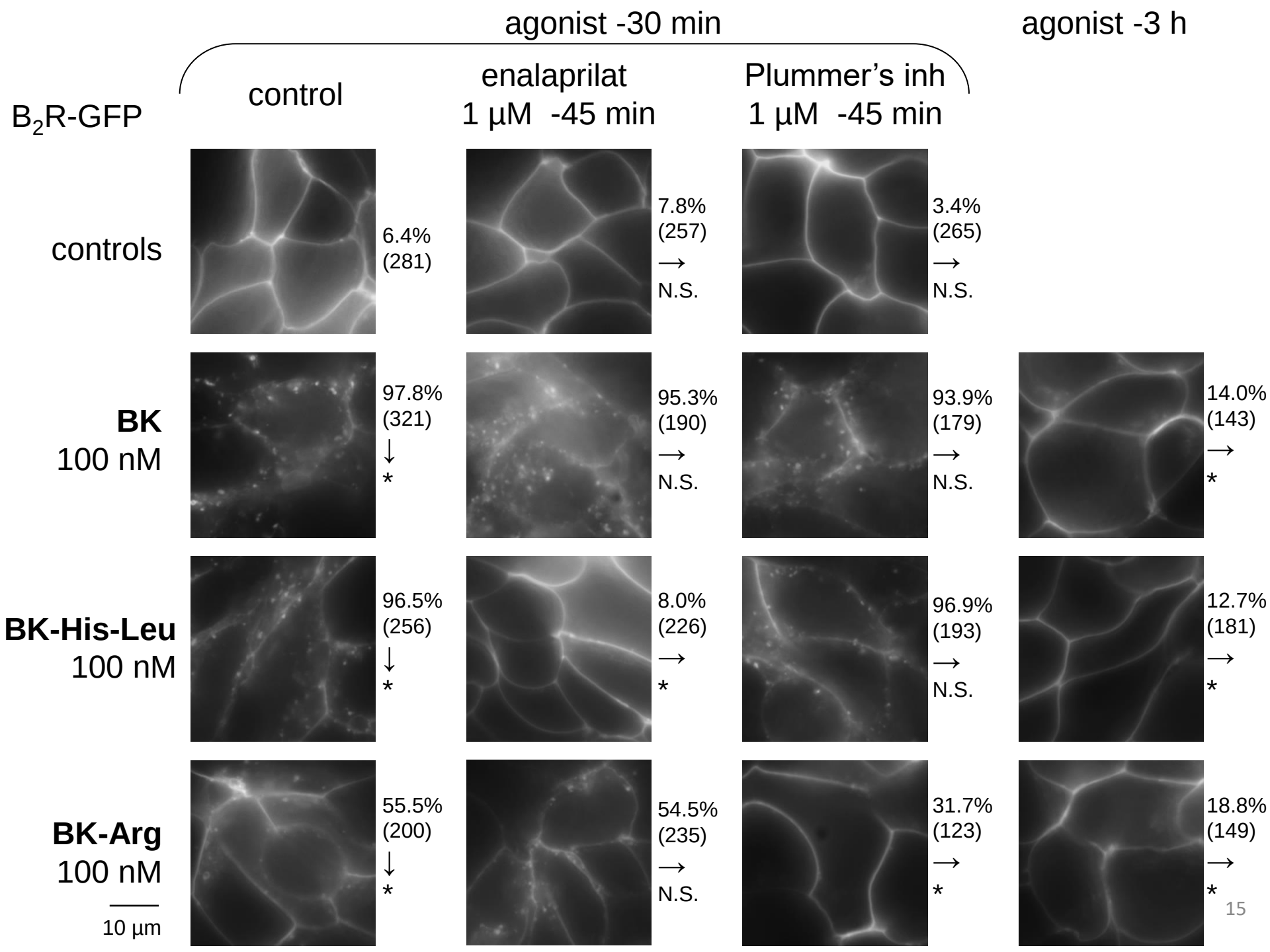


enalaprilat

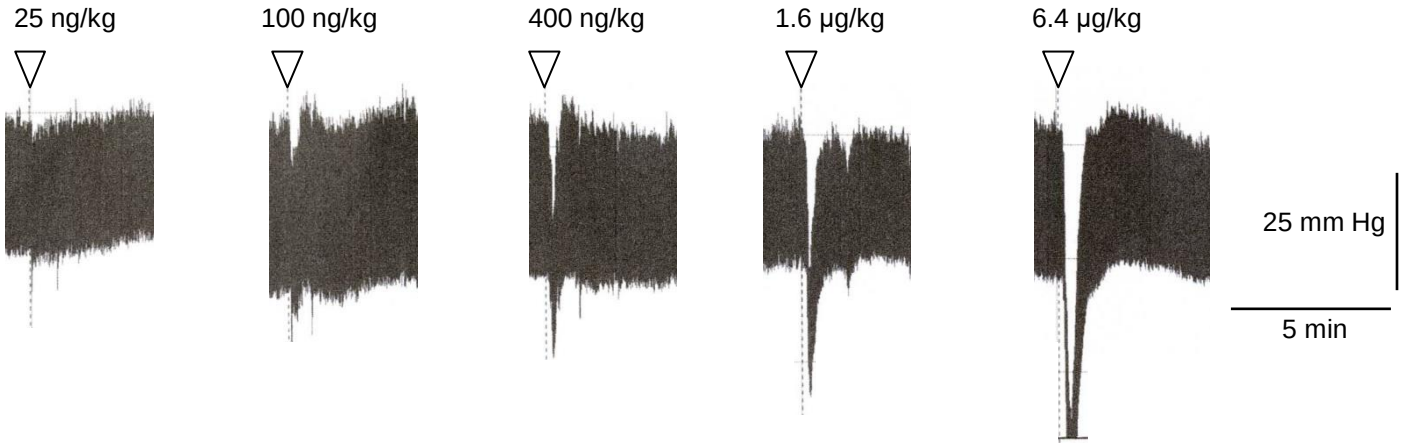

binding of 3 nM [3H]BK to B2R-GFP
n = 5-7
residual specific binding (%)
125
100
75
50
25
0
10-9
10-8
10-7
10-6
10-5
[unlabeled competitor] (M)
BK
BK-His-Leu

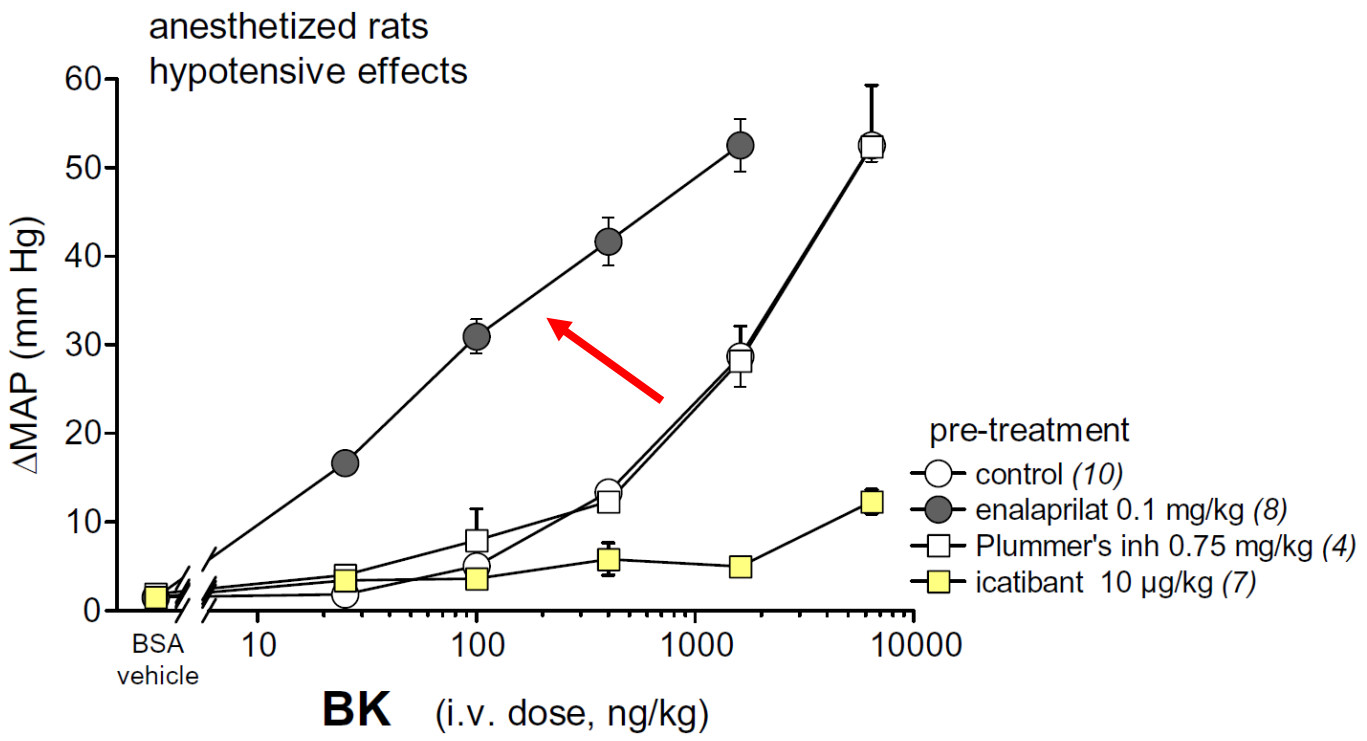

binding of 2 nM [3H]enalaprilat to ACE
n = 4-5
residual specific binding (%)
125
100
75
50
25
0
10-9
10-8
10-7
10-6
10-5
[unlabeled competitor] (M)
BK
BK-His-Leu


% maximal BK-induced contraction
Human umbilical vein contractility
100
75
50
25
0
10-9
10-8
10-7
10-6
10-5
[BK] (M)
control (8)
+ enalaprilat 1 µM (8)
Human umbilical vein contractility
[BK-His-Leu] (M)
control (8)
+ enalaprilat 1 µM (8)

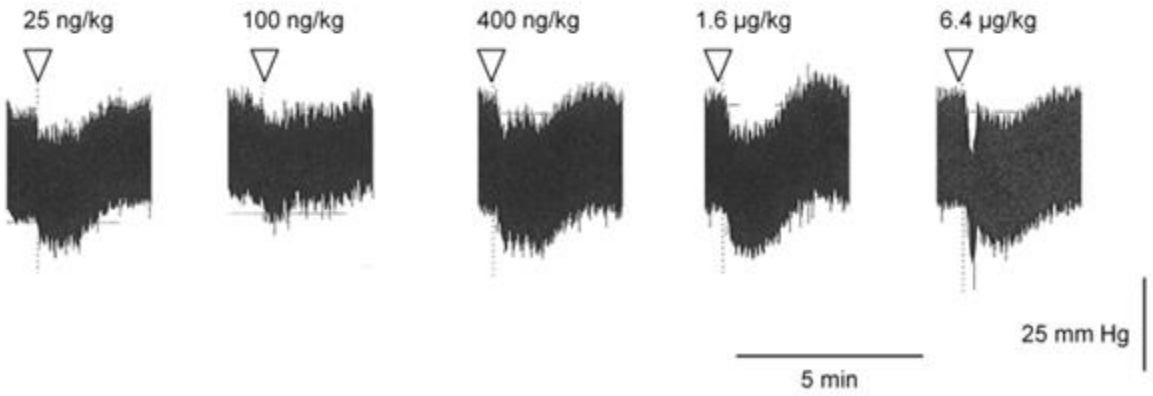

agonist -30 min
agonist -3 h
control
enalaprilat
1 µM -45 min
Plummer's inh
1 µM -45 min
B2R-GFP
controls
6.4%
(281)
7.8%
(257)
→
N.S.
3.4%
(265)
→
N.S.
BK
100 nM
97.8%
(321)
↓
*
95.3%
(190)
→
N.S.
93.9%
(179)
→
N.S.
14.0%
(143)
→
*
BK-His-Leu
100 nM
96.5%
(256)
↓
*
8.0%
(226)
→
*
96.9%
(193)
→
N.S.
12.7%
(181)
→
*
BK-Arg
100 nM
55.5%
(200)
↓
*
54.5%
(235)
→
N.S.
31.7%
(123)
→
*
18.8%
(149)
→
*
10 µm

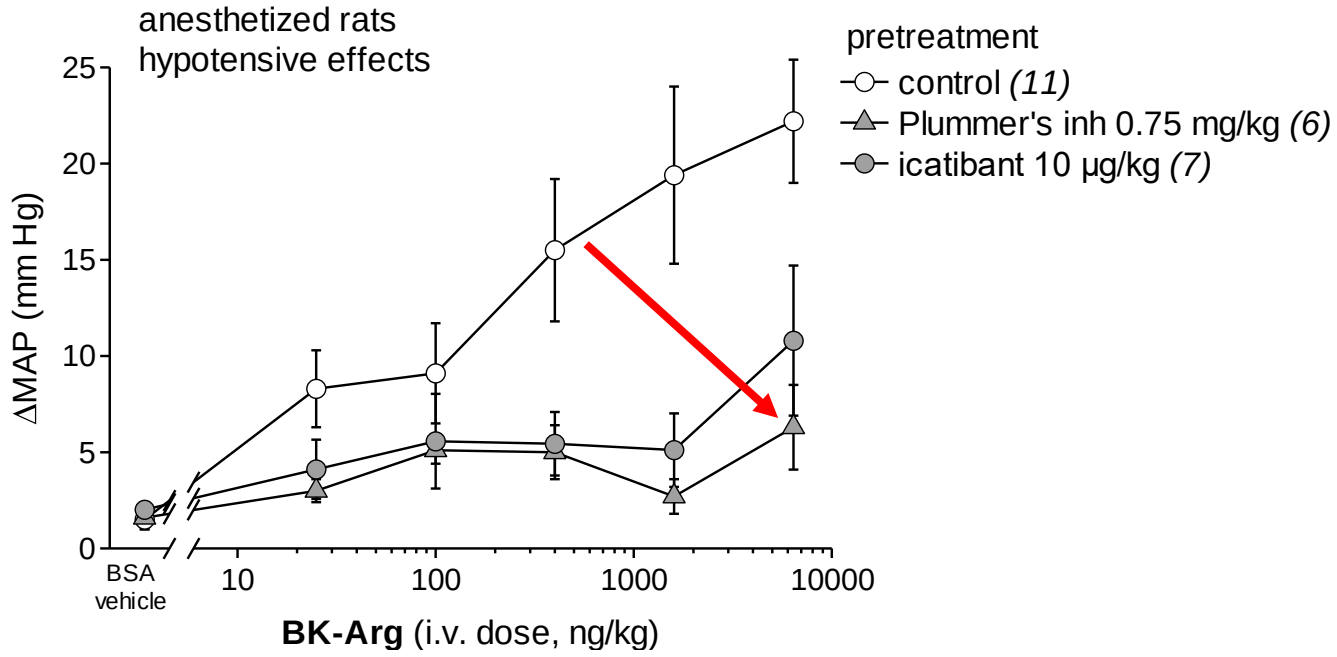
i.v. BK


25 ng/kg
100 ng/kg
400 ng/kg
1.6 µg/kg
6.4 µg/kg
25 mm Hg
5 min

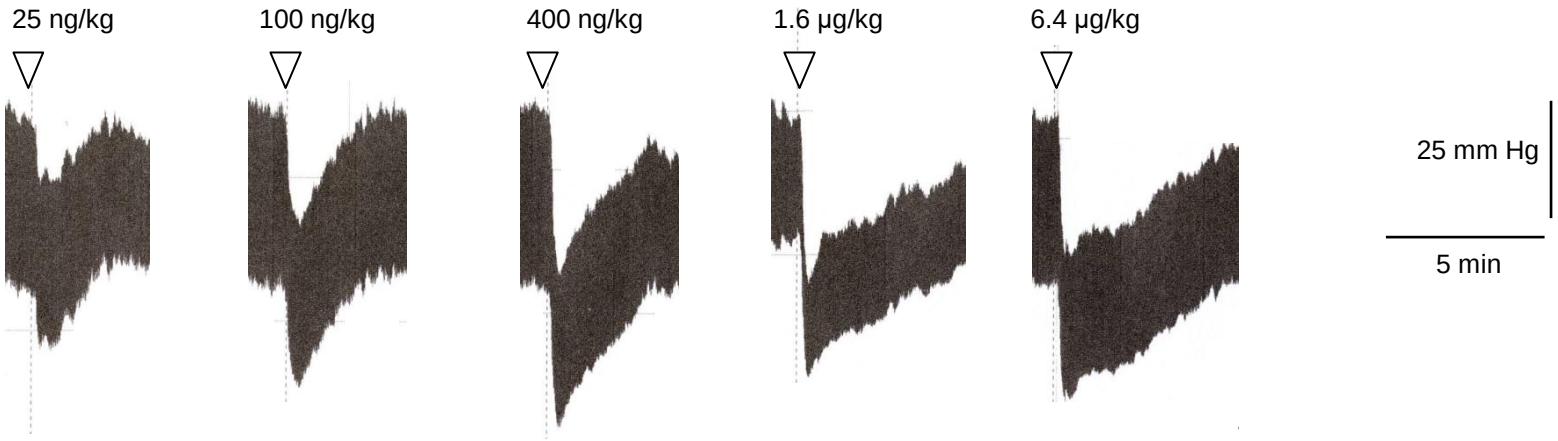

anesthetized rats
hypotensive effects
ΔMAP (mm Hg)
60
50
40
30
20
10
0
BSA
vehicle
10
100
1000
10000
BK (i.v. dose, ng/kg)
pre-treatment
control (10)
enalaprilat 0.1 mg/kg (8)
Plummer's inh 0.75 mg/kg (4)
icatibant 10 µg/kg (7)

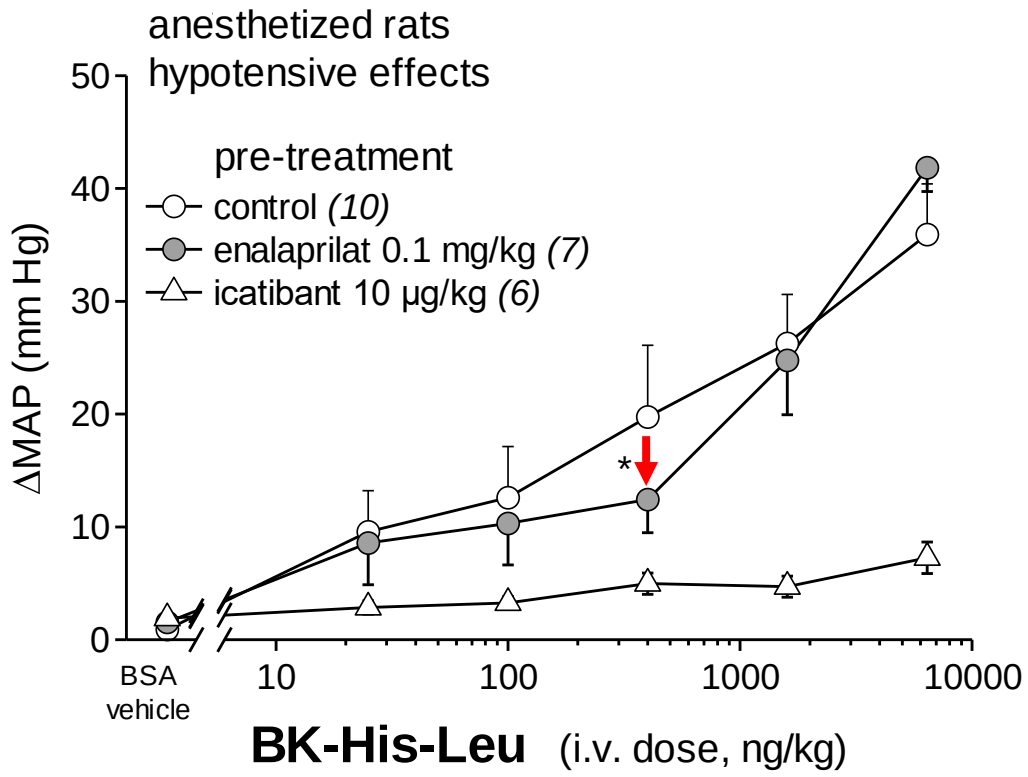
BK-Arg


25 ng/kg
100 ng/kg
400 ng/kg
1.6 µg/kg
6.4 µg/kg
25 mm Hg
5 min


anesthetized rats
hypotensive effects
pretreatment
control (11)
Plummer's inh 0.75 mg/kg (6)
icatibant 10 µg/kg (7)
ΔMAP (mm Hg)
25
20
15
10
5
0
BSA
vehicle
10
100
1000
10000
BK-Arg (i.v. dose, ng/kg)

BK-His-Leu


25 ng/kg
100 ng/kg
400 ng/kg
1.6 µg/kg
6.4 µg/kg
25 mm Hg
5 min


anesthetized rats
hypotensive effects
pre-treatment
control (10)
enalaprilat 0.1 mg/kg (7)
icatibant 10 µg/kg (6)
ΔMAP (mm Hg)
50
40
30
20
10
0
*
BSA
vehicle
10
100
1000
10000
BK-His-Leu (i.v. dose, ng/kg)

Conclusions

Vascular ectopeptidases were shown to activate latent agonists of the H1 and B2 receptors.

The concept could be extended to various classes of drugs for a local action on the vasculature.

The selection of the activating vascular peptidase is critical for the selectivity of this line of investigation.

Prodrugs of the short-lived peptide BK, exploiting vascular and blood plasma peptidases to stimulate the most desirable effects of endothelial B2 receptors might find application in intensive care situations where an intravenous line is available (unstable angina, myocardial infarction, perhaps decompensated congestive heart failure) and possibly, in more chronic ailments (e.g., pulmonary hypertension).

Acknowledgments

funding

- Dr. Hélène Bachelard
- Dr. Lajos Gera
- Dr. Xavier Charest-Morin
- Ms. Johanne Bouthillier
- Ms. Melissa Jean

IRSC CIHR
Instituts de recherche en santé du Canada
Canadian Institutes of Health Research

Fonds de recherche Santé Québec