



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



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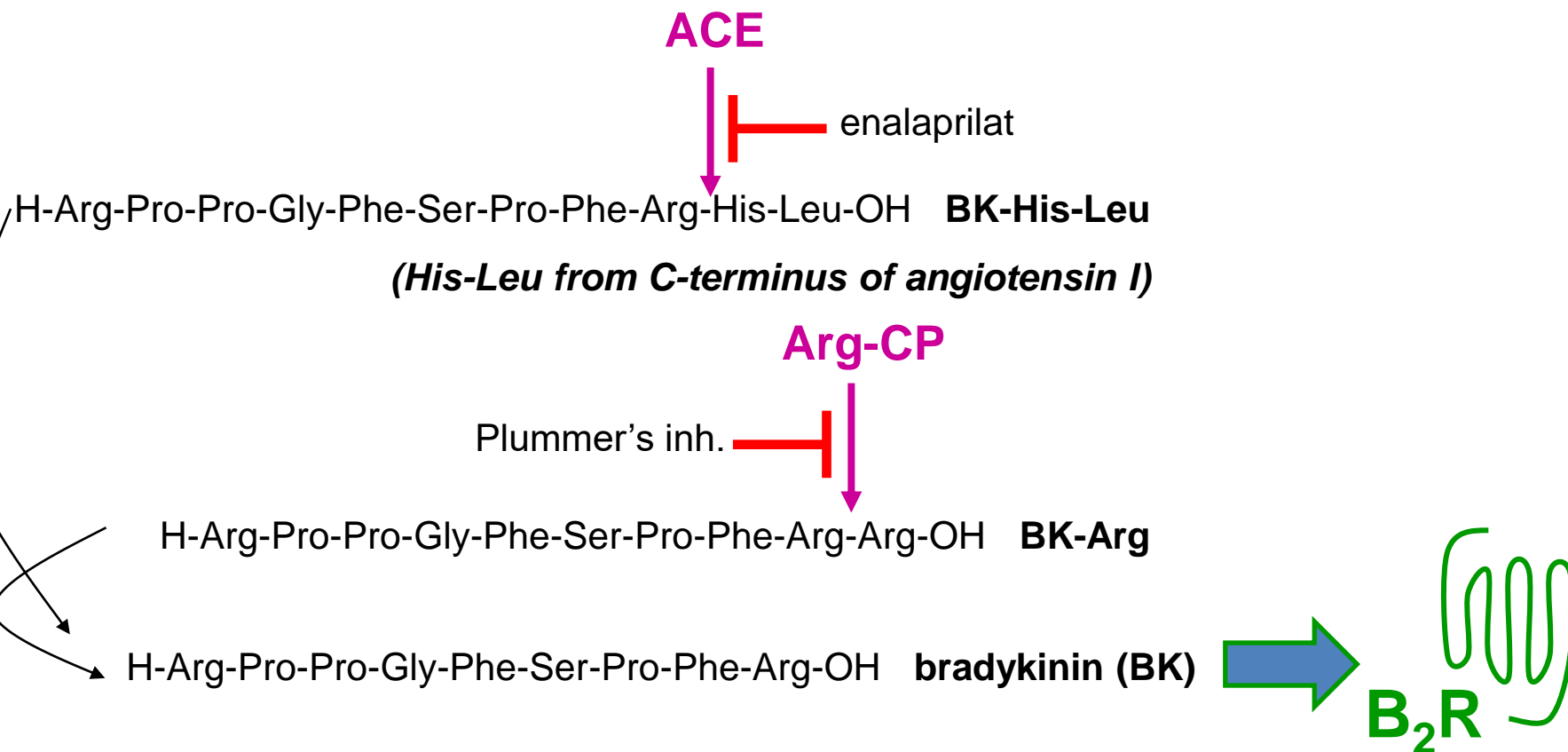
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Infectieuses &
Immunitaires**

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, Arg⁰-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.

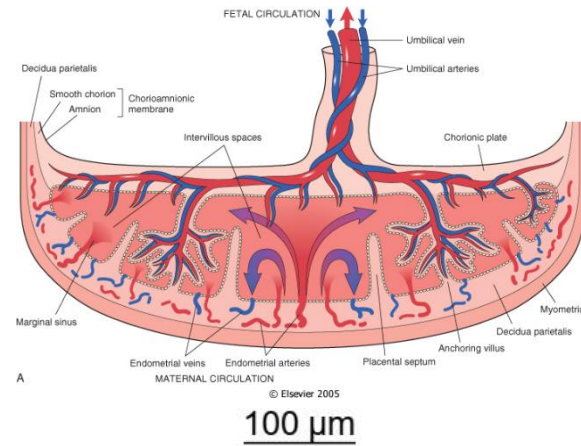
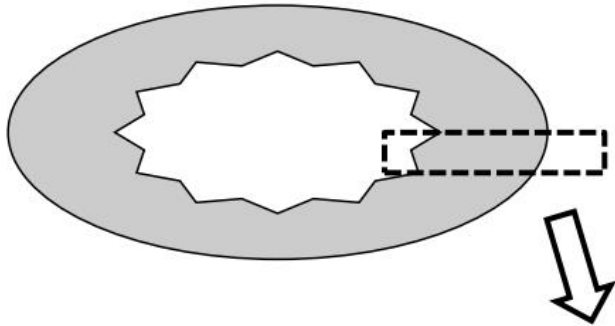
Introduction

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

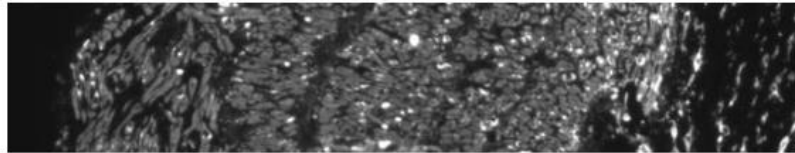
A “prodrug” strategy where a vasoactive agonist is activated only at the level of the microcirculation by resident peptidases is presented.

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.

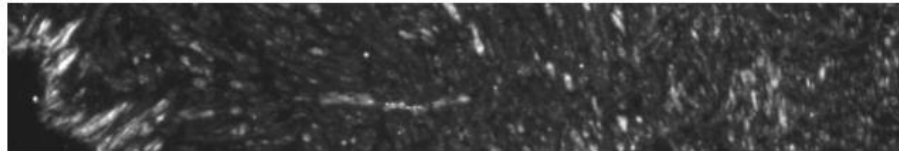
human umbilical vein



α-actin monoclonal



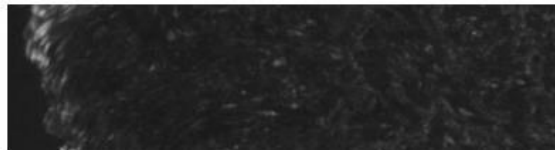
APN (CD13)



2nd anti-mouse Ab

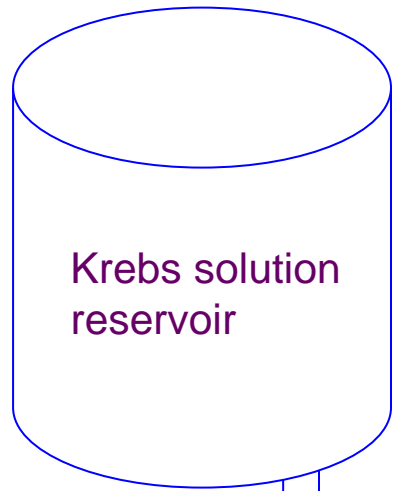
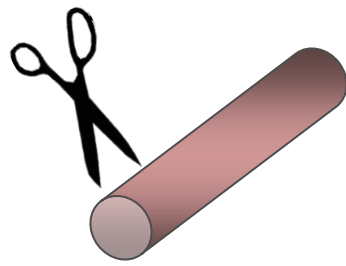


ACE polyclonal

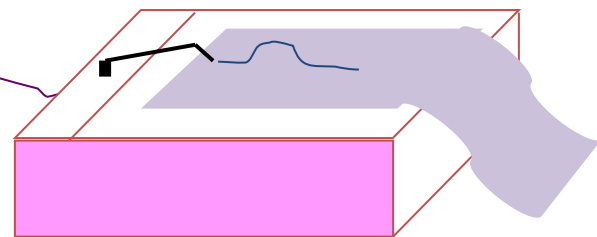
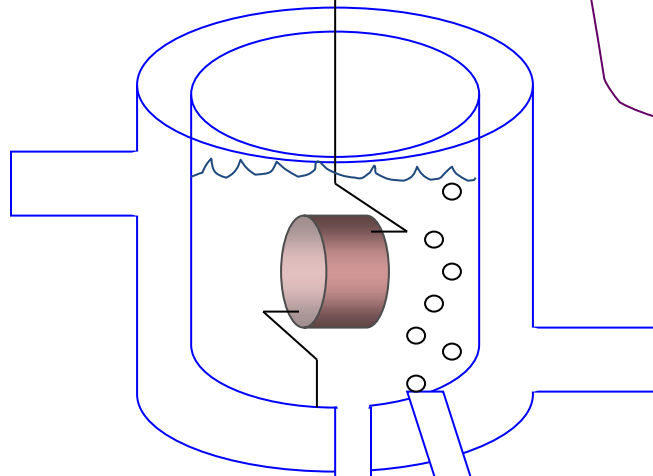


2nd anti-rabbit Ab





Force transducer



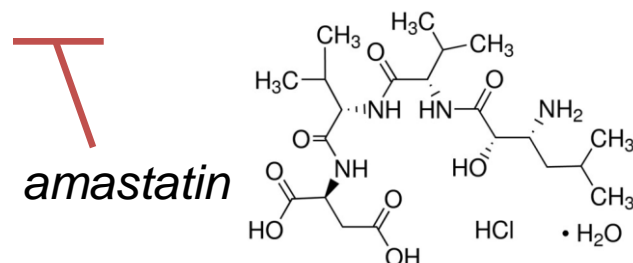
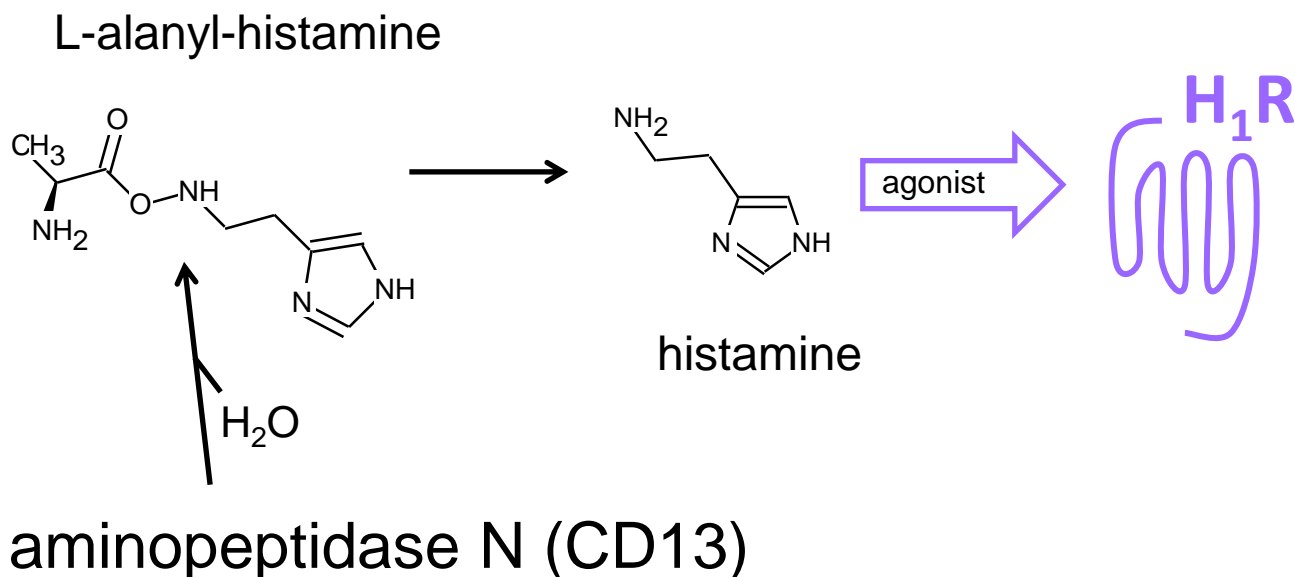
95% O₂ + 5% CO₂



drain



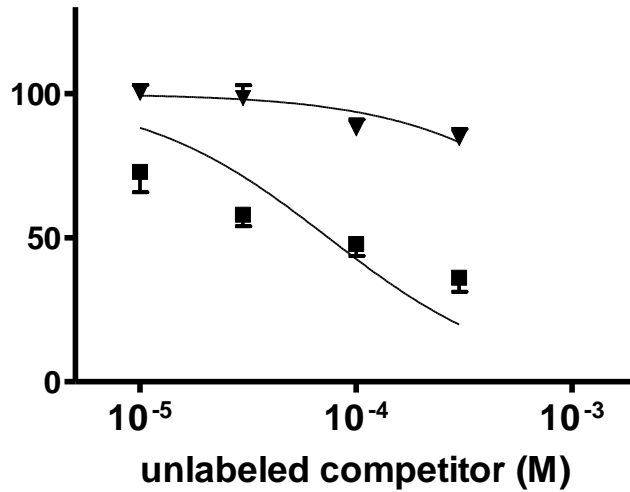
1. A primary amine released by vascular aminopeptidase N



Int. Immunopharmacol.
2013; 17: 677

hH₁R (n = 5-6)

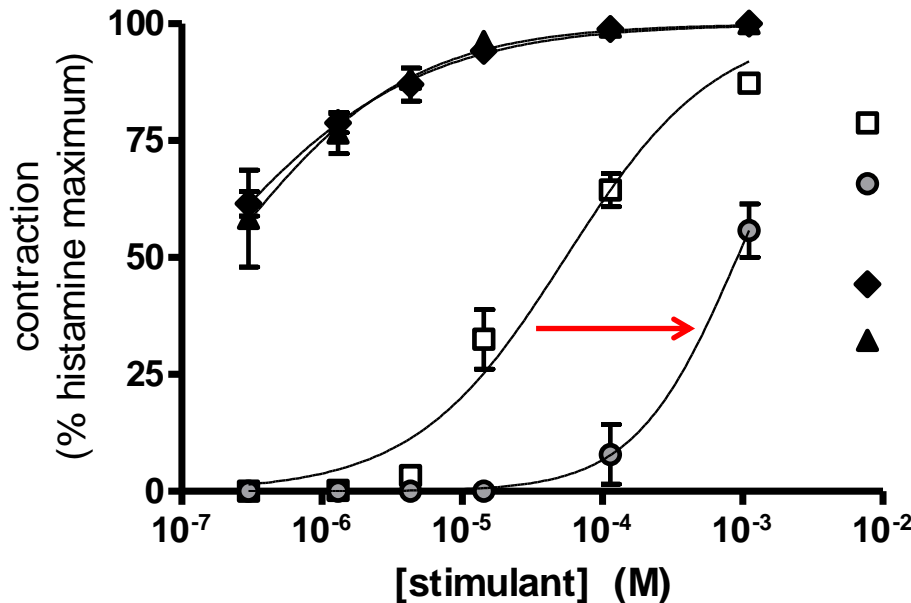
residual specific binding (%)



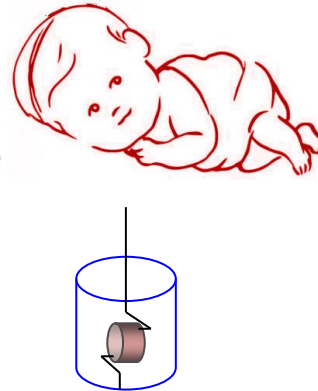
- histamine
- ▼ L-alanine-histamine

Binding competition of [³H]pyrilamine (2 nM) to human recombinant H₁R

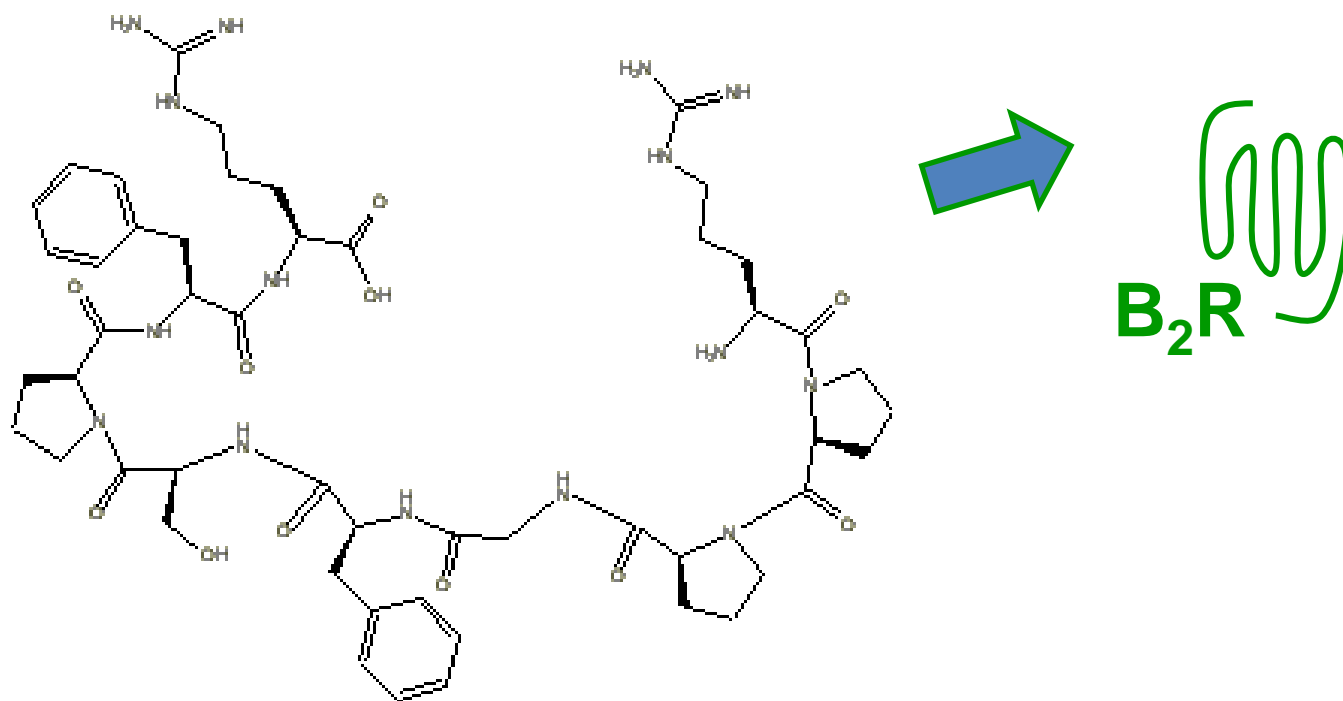
Umbilical vein contractility



- saline / L-Ala-histamine
- amastatin 3 μM / L-Ala-histamine
- ◆ saline / histamine
- ▲ amastatin 3 μM / histamine

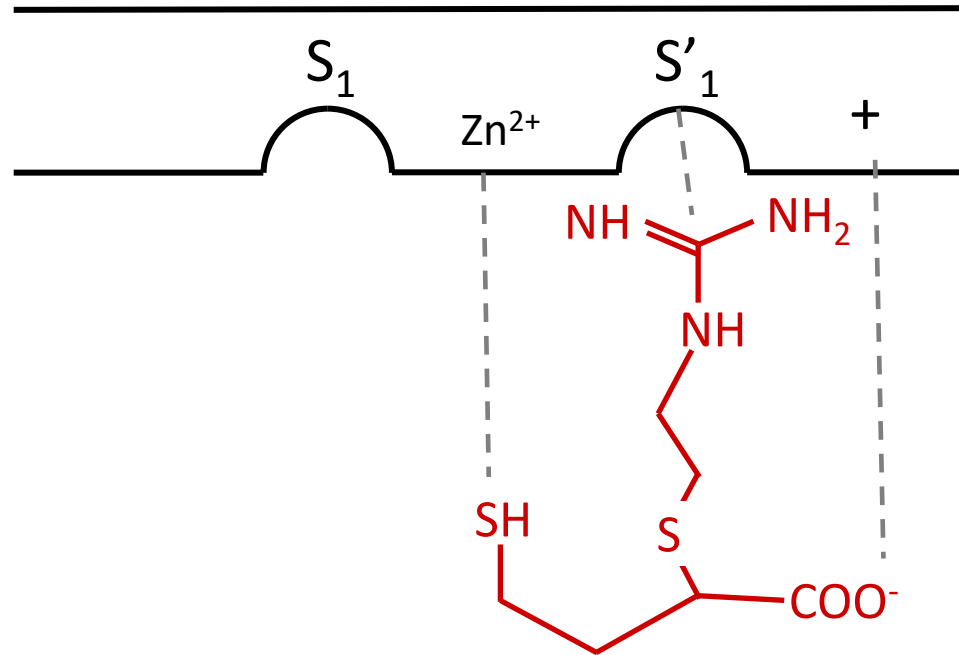


2. Extended bradykinin sequences activated by arginine-carboxypeptidases (kininase I) and angiotensin converting enzyme (kininase II)

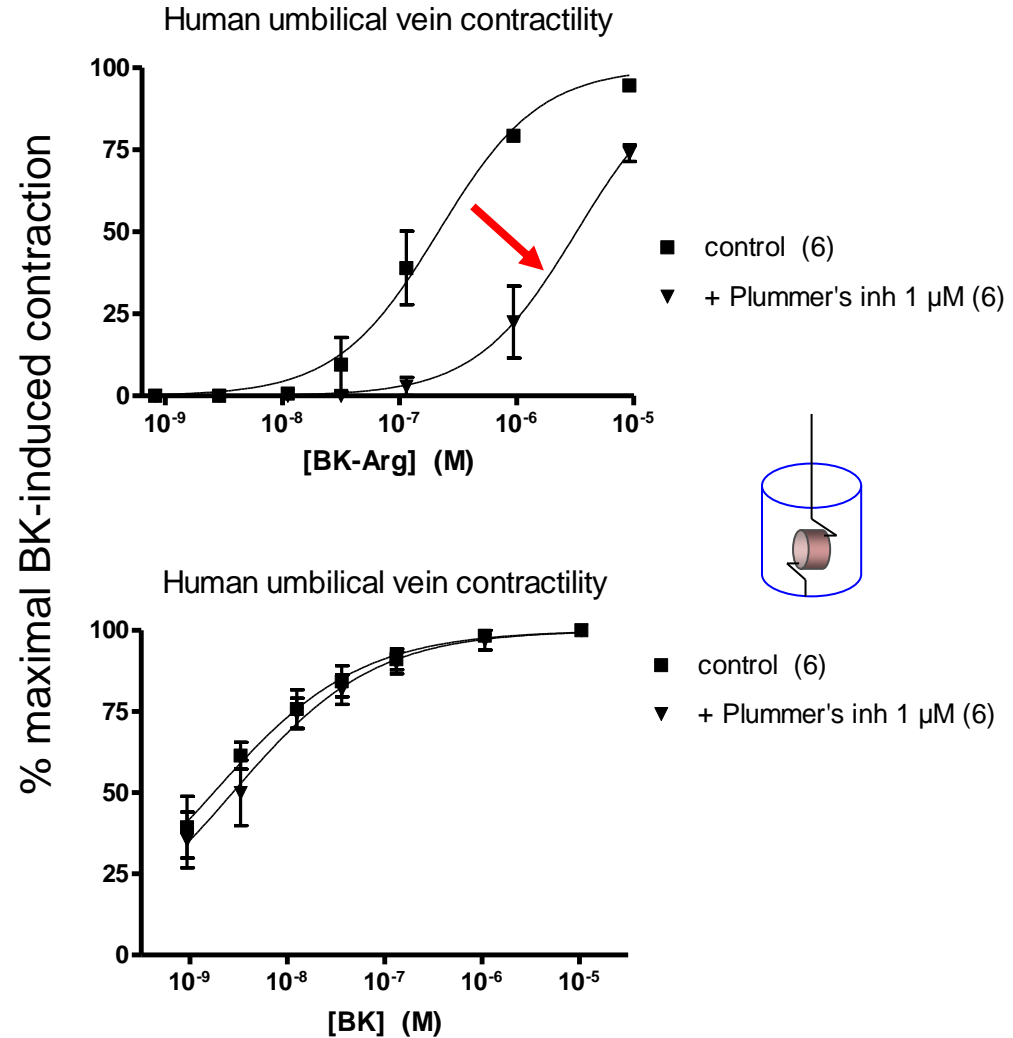
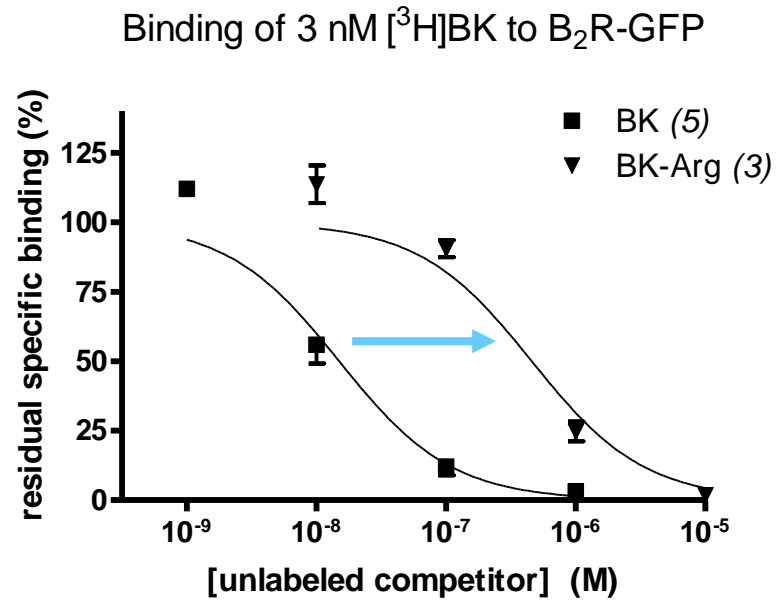


Front. Pharmacol.
2014; 5: 32
2016; 6: 306
2018; 9: 273

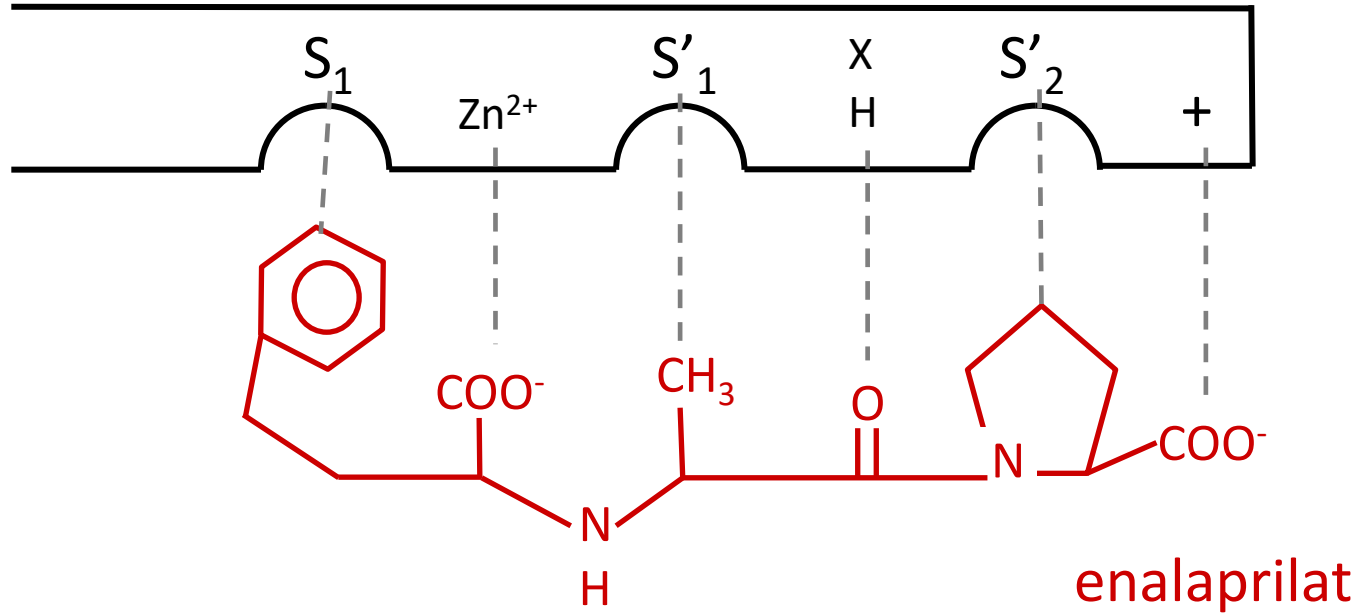
Arg-CP



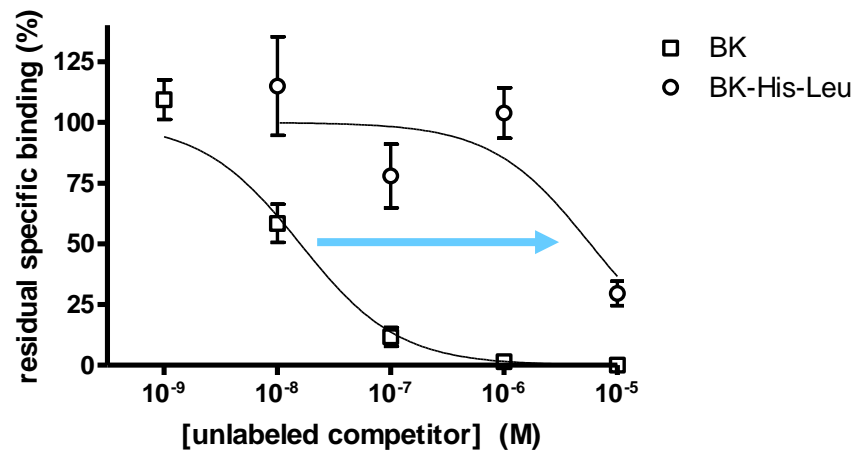
Plummer's inh



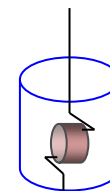
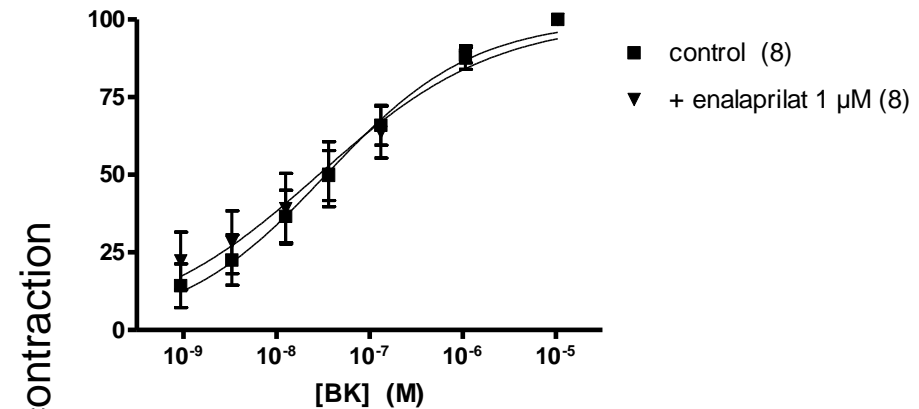
ACE



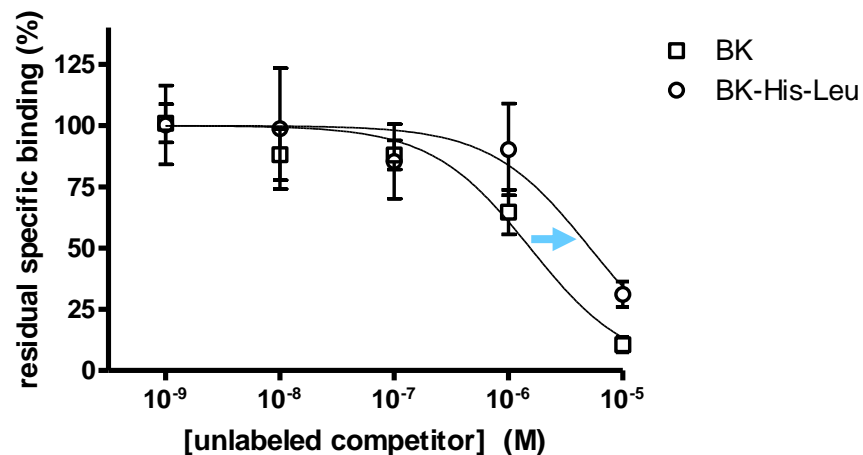
binding of 3 nM [3 H]BK to B₂R-GFP
n = 5-7



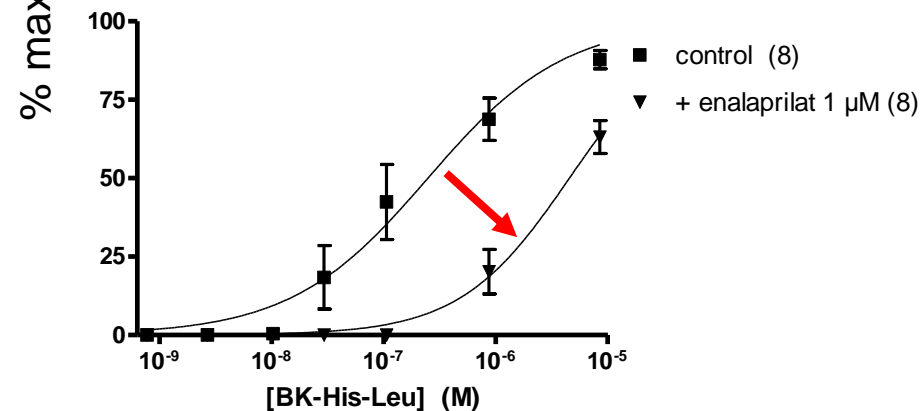
Human umbilical vein contractility

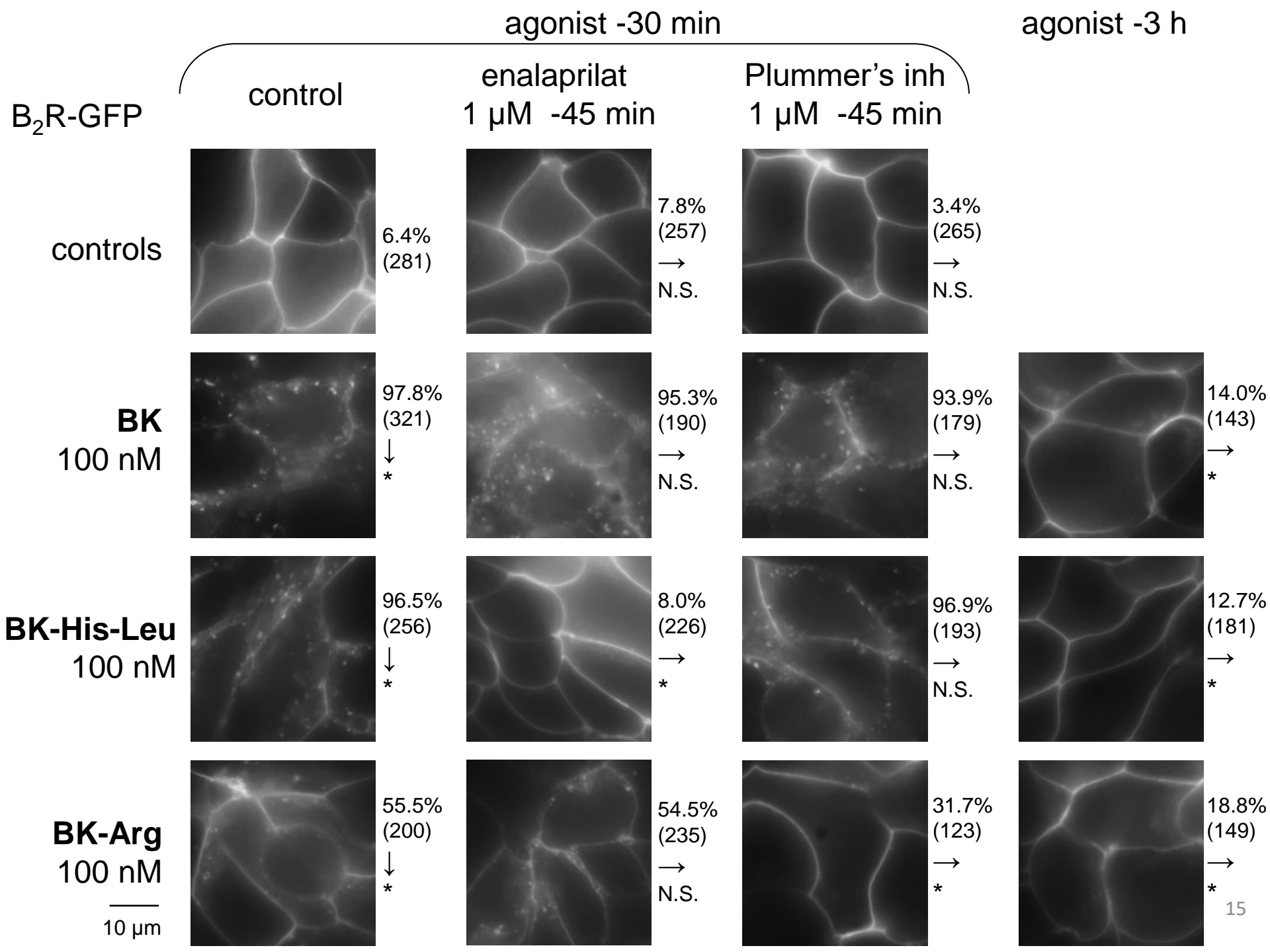


binding of 2 nM [3 H]enalaprilat to ACE
n = 4-5

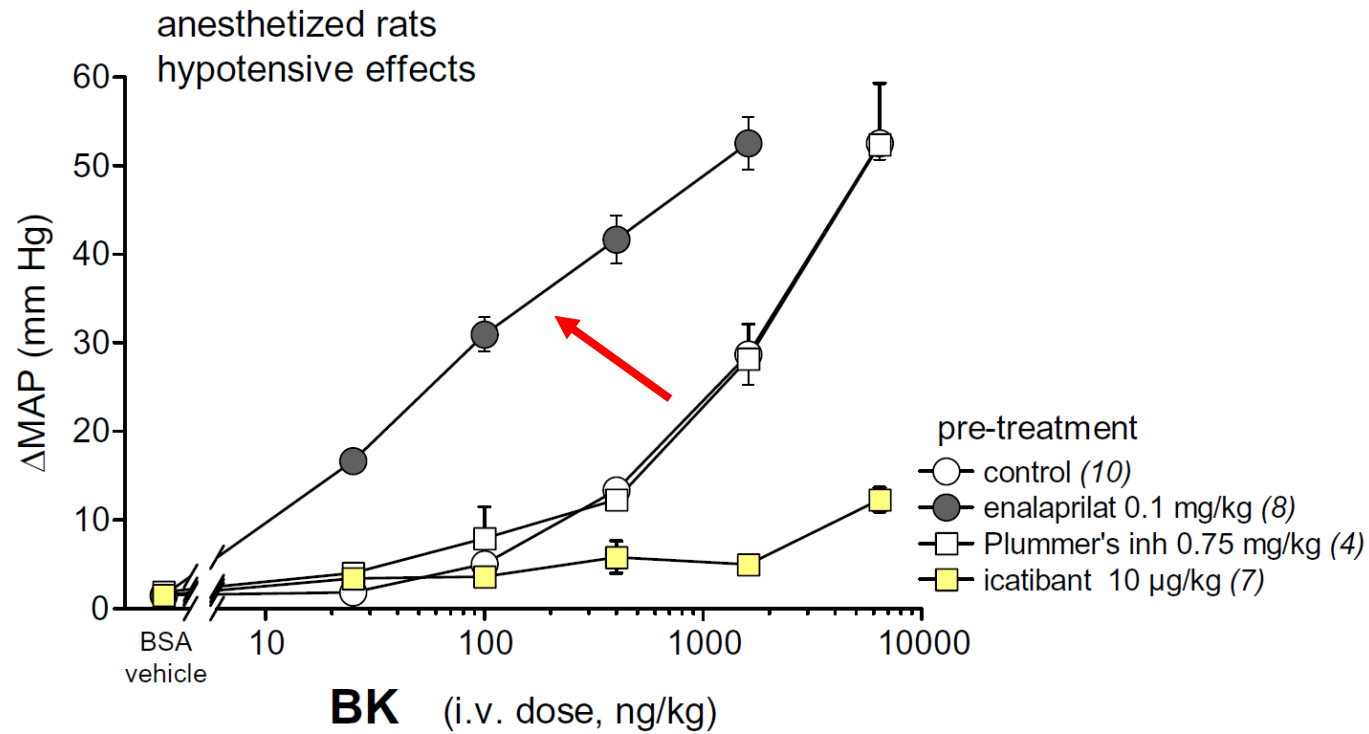
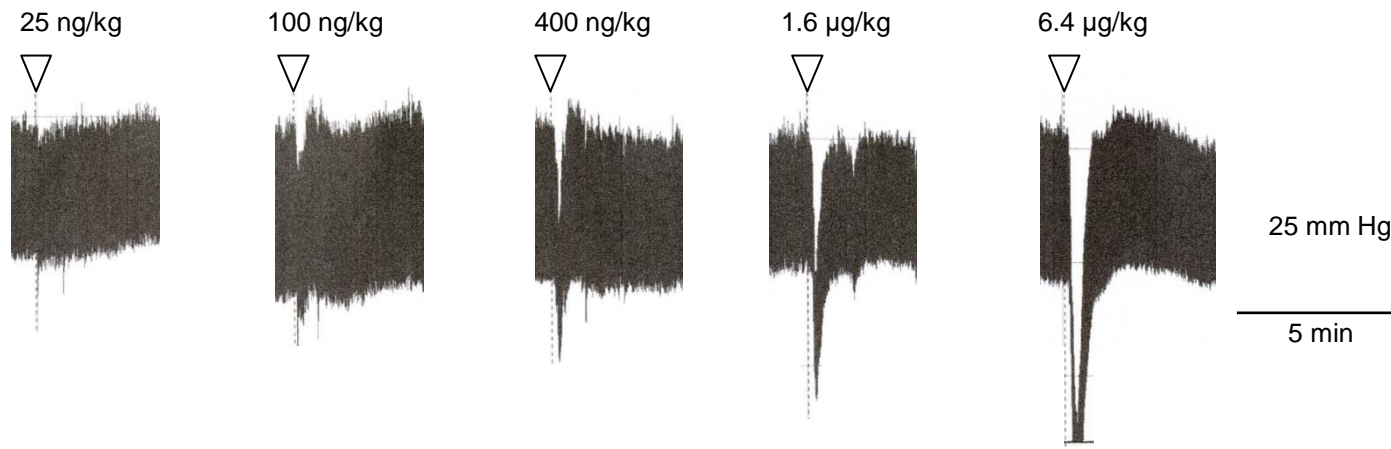


Human umbilical vein contractility

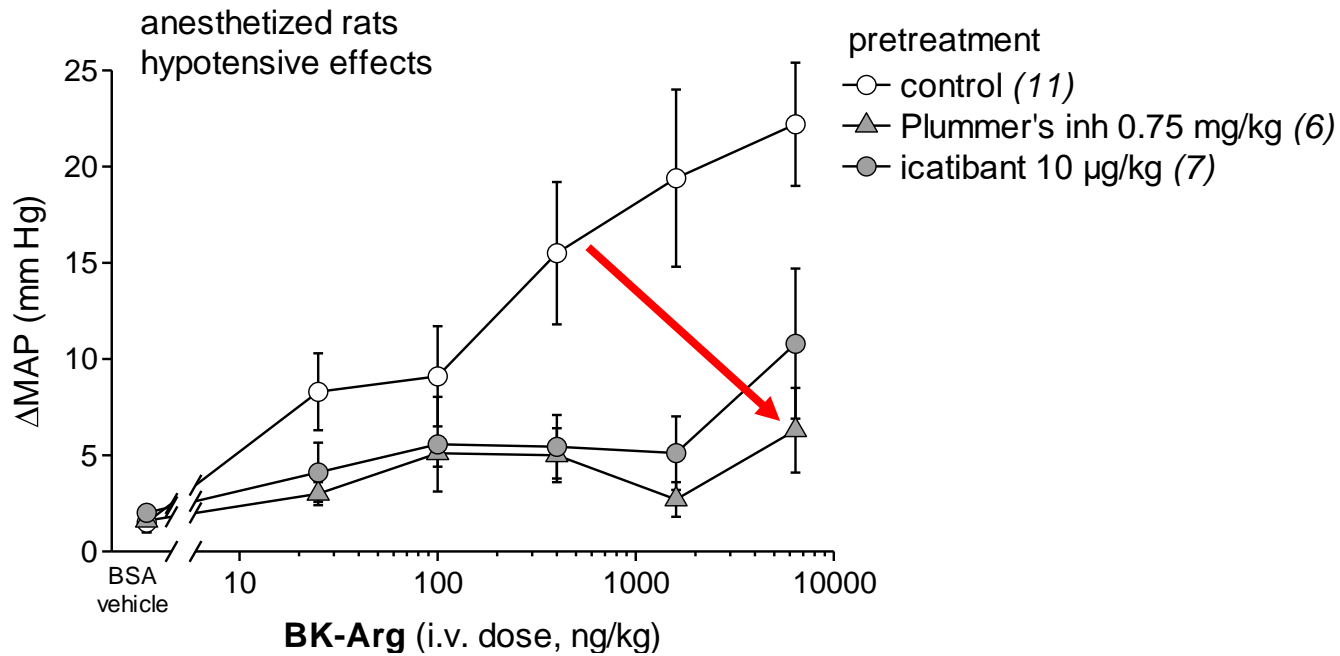
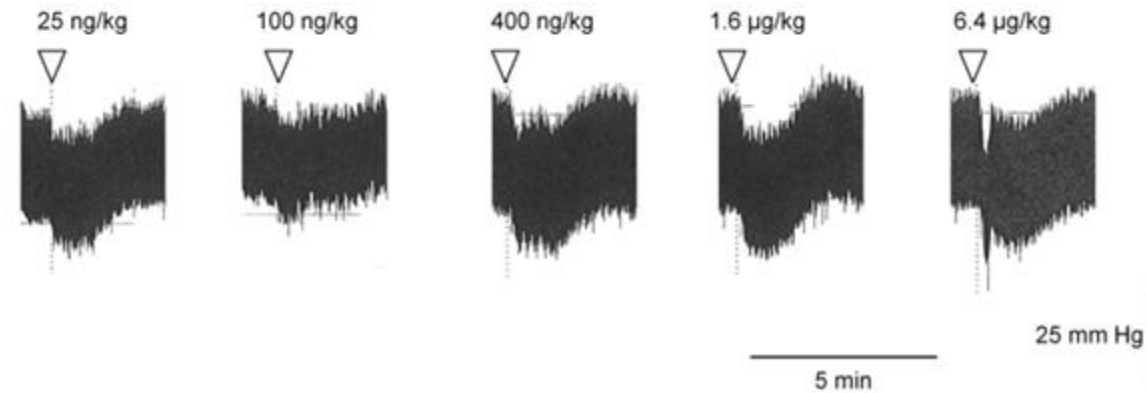




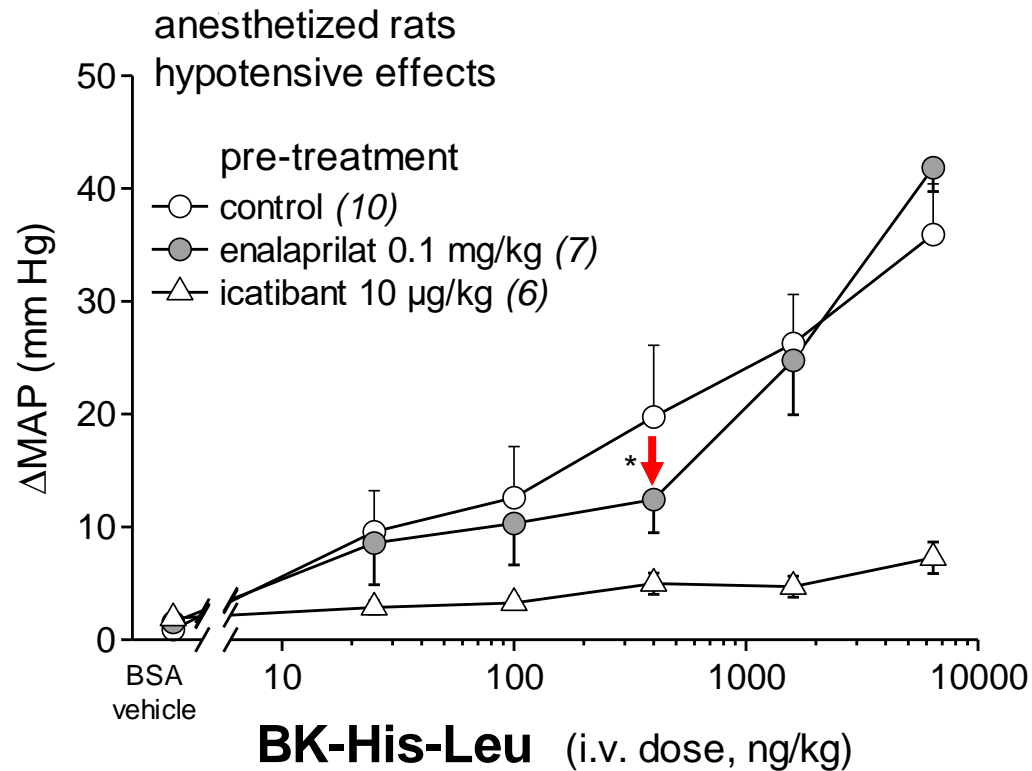
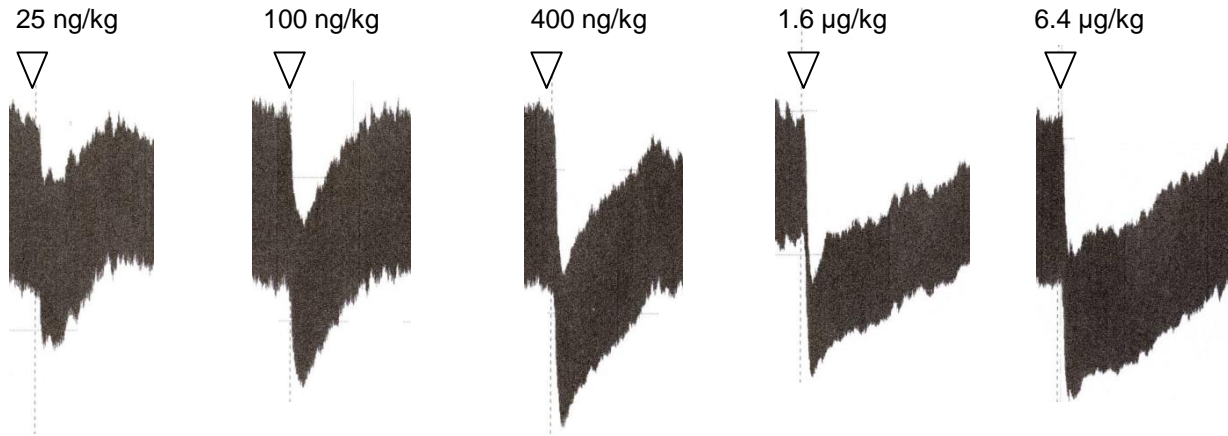
i.v. BK



BK-Arg



BK-His-Leu



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

- Dr. H  l  ne Bachelard
- Dr. Lajos Gera
- Dr. Xavier Charest-Morin
- Ms. Johanne Bouthillier
- Ms. Melissa Jean

funding



IRSC **CIHR**
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