A robust bioassay of the human bradykinin B_2 receptor that extends molecular/cellular studies: the isolated umbilical vein

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Centre de recherche
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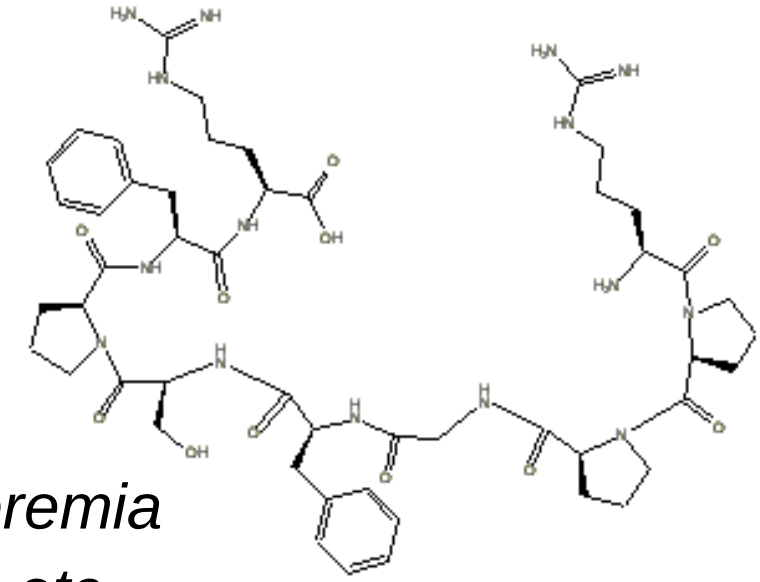
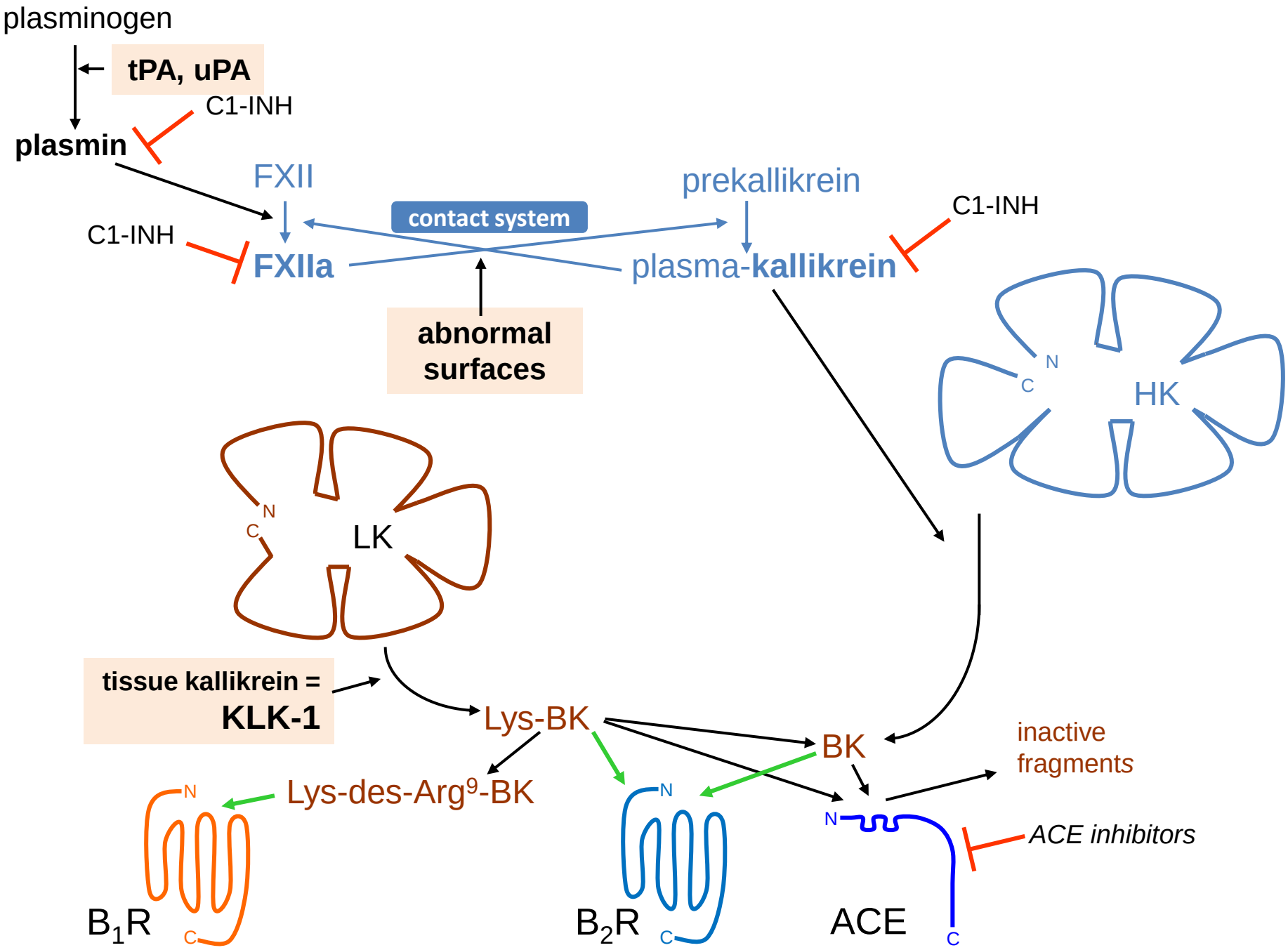
Importance of bradykinin (BK)

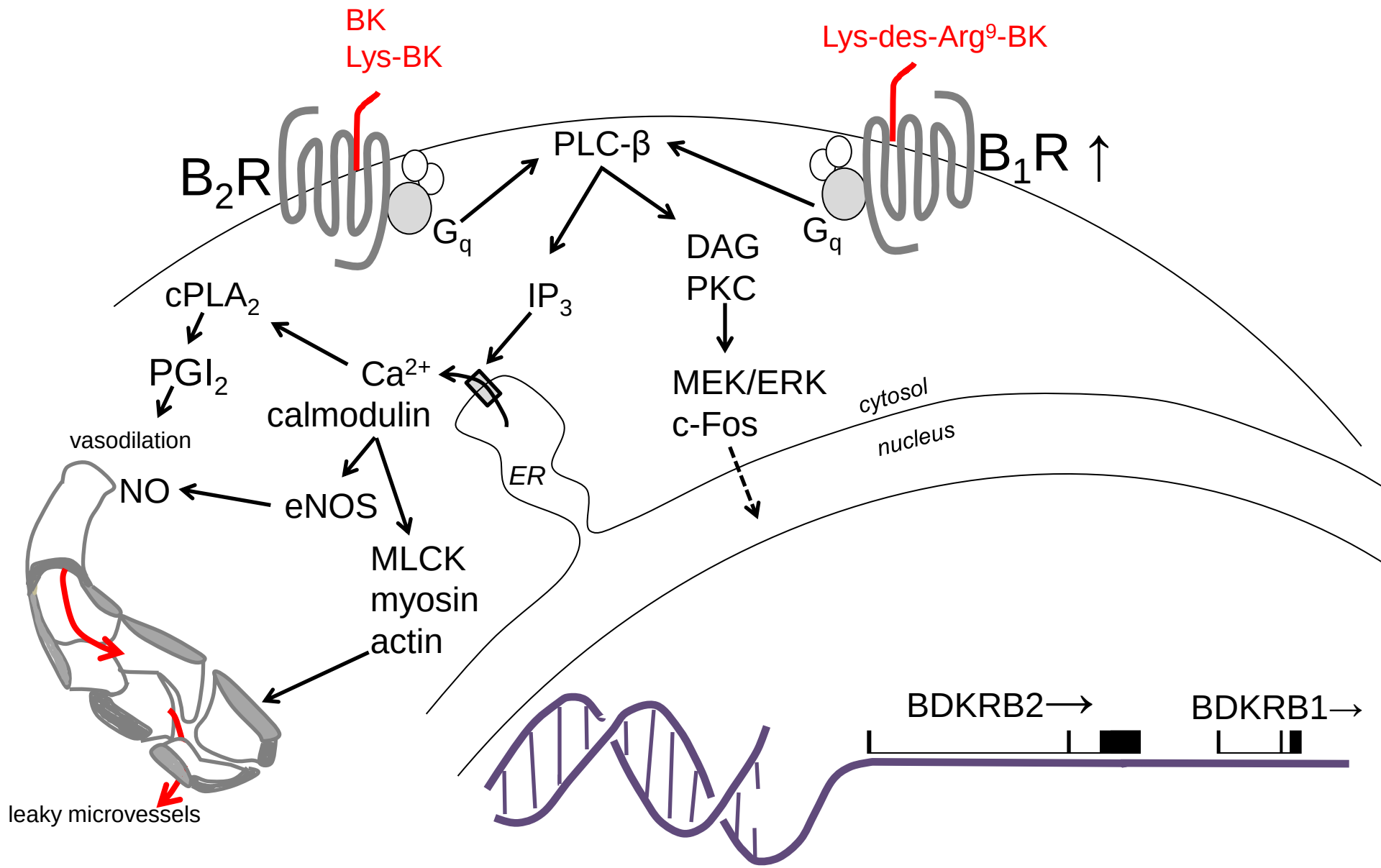
- Derived from kininogens via the action of kallikreins
- A small and unstable peptide



- Target cell types:
  - Endothelial cells: *edema, hyperemia*
  - Sensory nerve terminals: *pain, etc.*
  - Epithelial cells: *various inflammatory consequences*
  - Smooth muscle cells


plasminogen
tPA, uPA
C1-INH
plasmin
FXII
C1-INH
FXIIa
contact system
prekallikrein
C1-INH
plasma-kallikrein
abnormal surfaces
N
C
HK
N
C
LK
tissue kallikrein = KLK-1
Lys-BK
BK
inactive fragments
Lys-des-Arg9-BK
N
N
ACE inhibitors
B1R
C
B2R
C
ACE
C

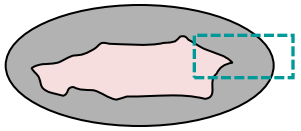
Signaling in endothelial cells



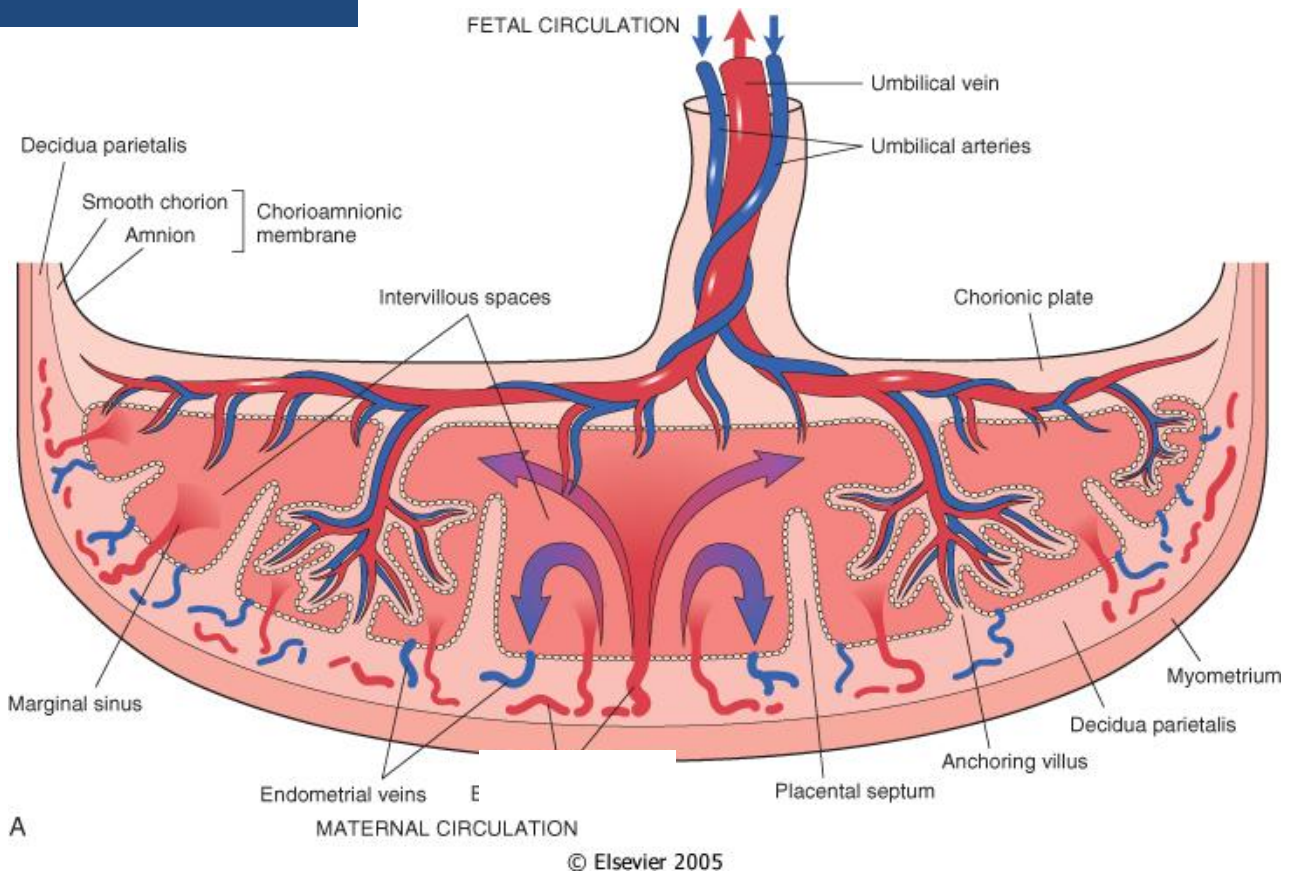
Need for a human bioassay

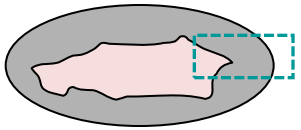
- In vitro screening of new ligands based on radioligand binding competition, simple signaling (e.g., intracellular calcium)
- BK B2R has a notoriously species-specific pharmacological profile
- Naturally expressed BK B2R in the umbilical vein (not overexpressed)

- B2R antagonists exert species-specific effect: need for a bioassay based on human tissues
- Stable in a time scale of hours



vein

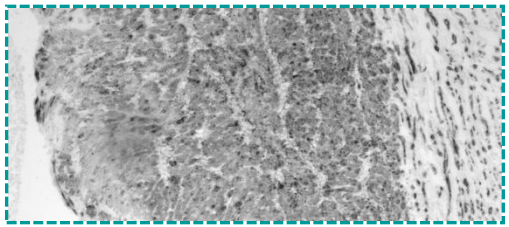




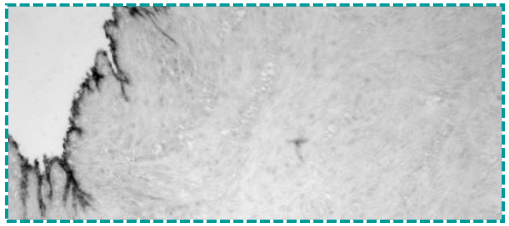
vein

- Obtained after uncomplicated elective caesarean sections (with informed consent)
- SMCs dominate → contractile response mediated by BK B_2Rs
- HUVECs: this is where they reside

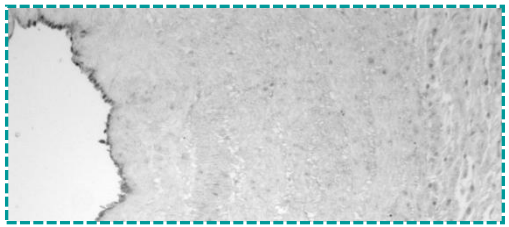
α-actin monoclonal



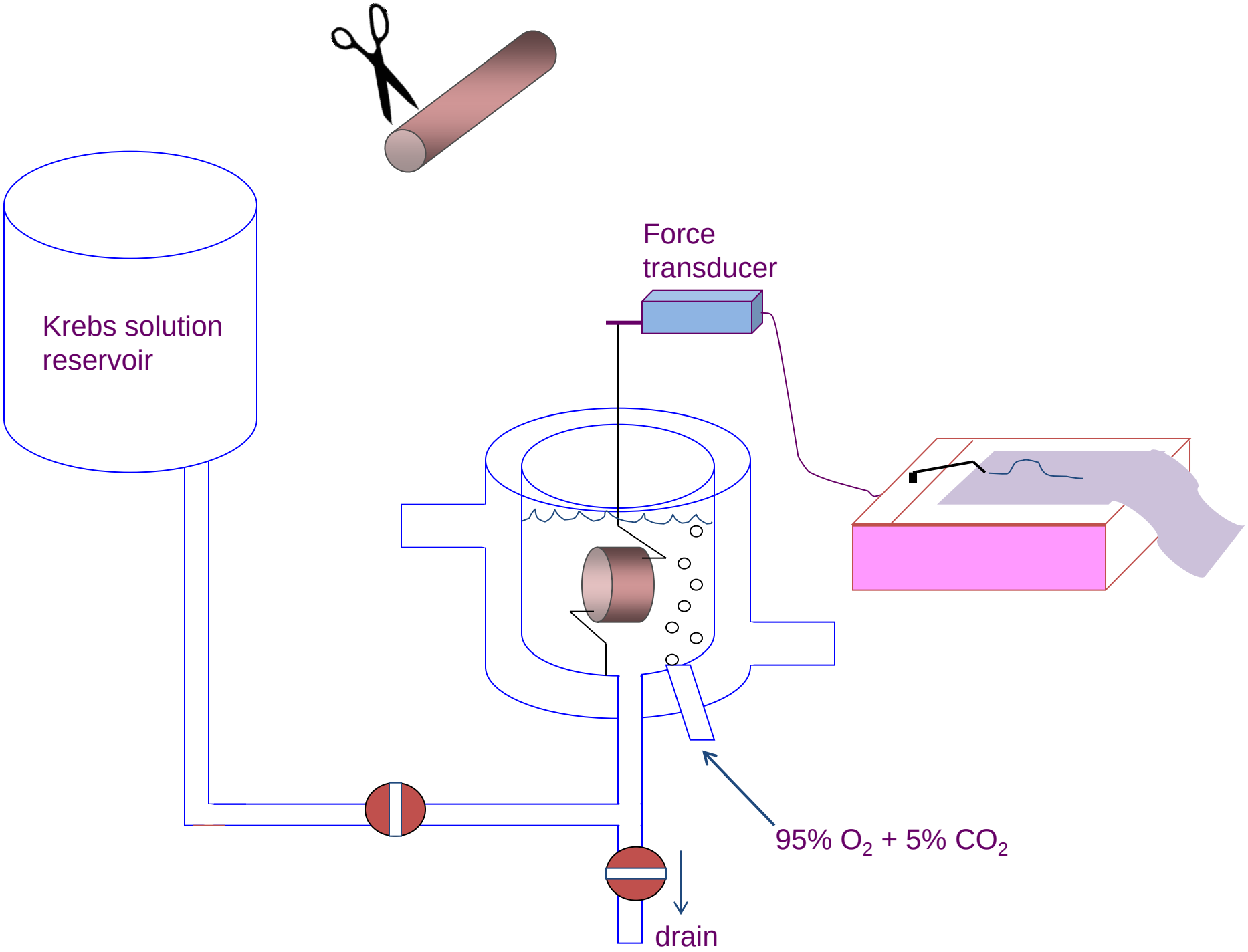
vWF polyclonal

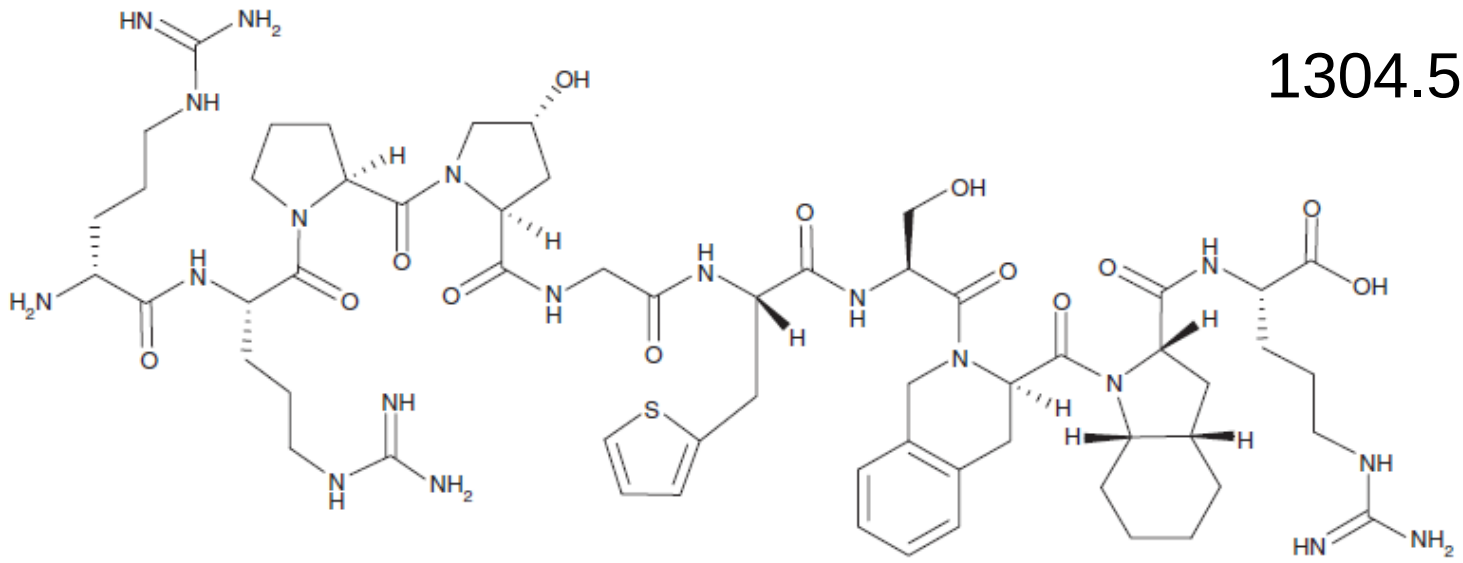


ACE (C28) polyclonal



Koumbadinga et al. *Peptides* 2010; **31**: 1546

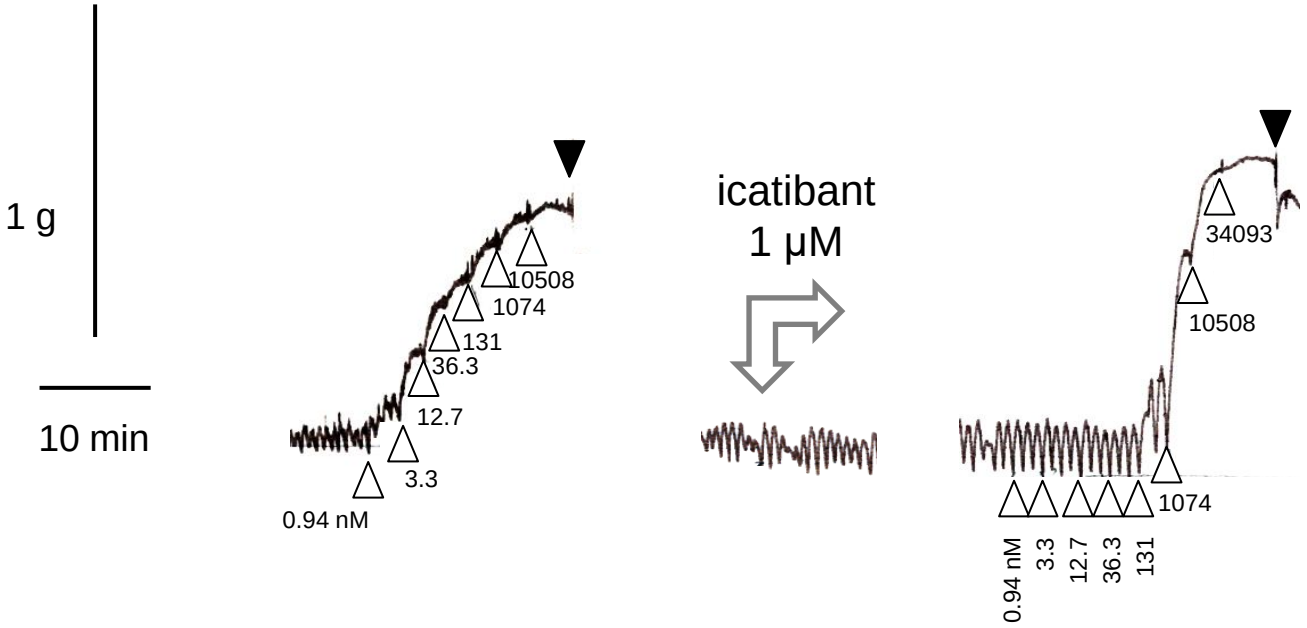

Krebs solution reservoir
Force transducer
95% O_2 + 5% CO_2
drain



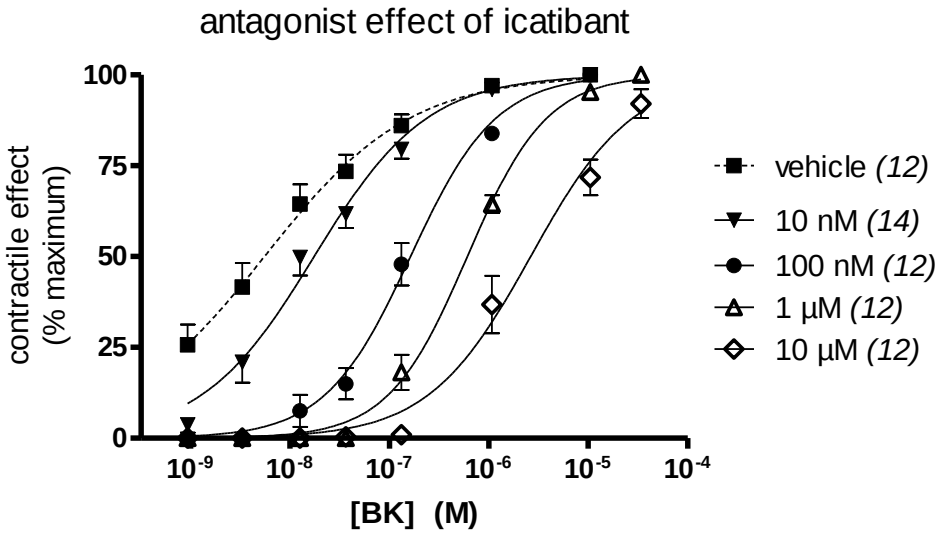
1304.5 g/mol

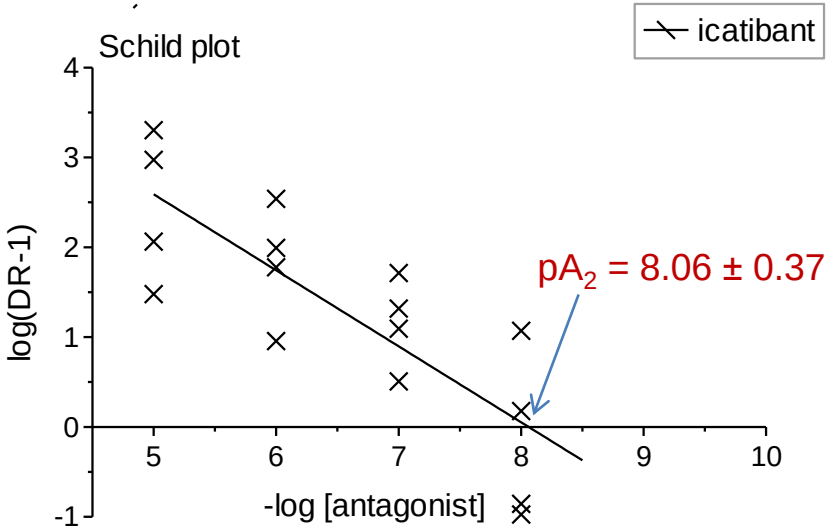
icatibant (Hoe 140; Firazyr; D-Arg[Hyp^3, Thi^5, D-Tic^7, Oic^8]-bradykinin)
reported by Hock et al., Br J Pharmacol 1991; 102, 769

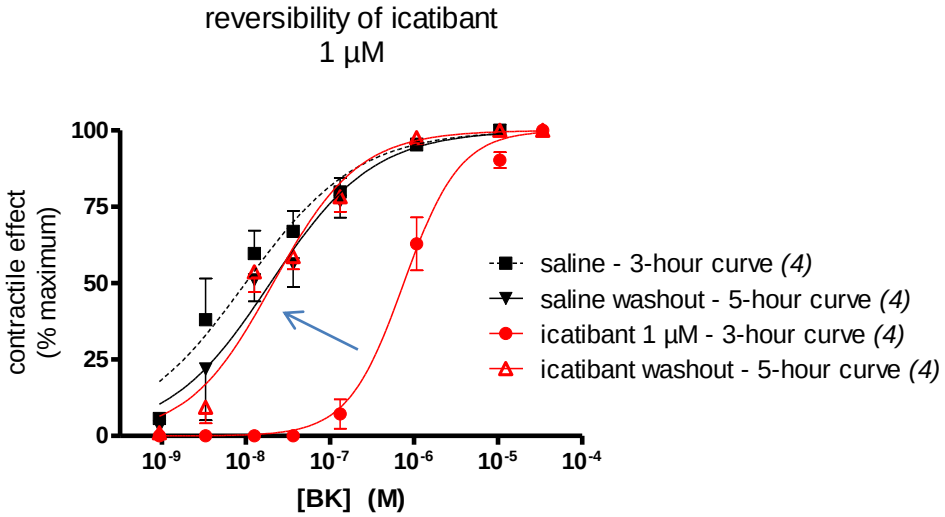
clinical use: auto-injected s.c., hereditary angioedema



Lesage et al., *Front. Pharmacol.* 2020; **11**: 916



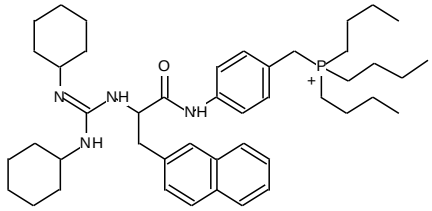


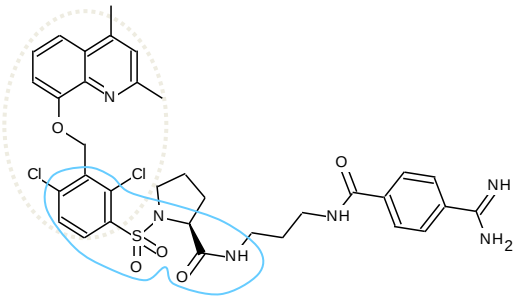


Lesage et al., *Front. Pharmacol.* 2020; **11**: 916

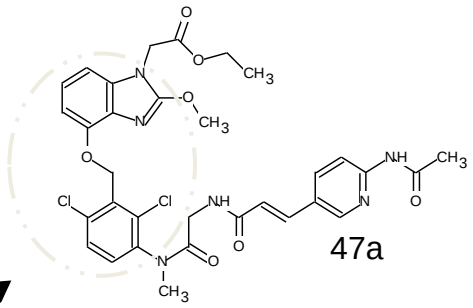
Small molecule antagonists of the BK B2R

WIN 64338

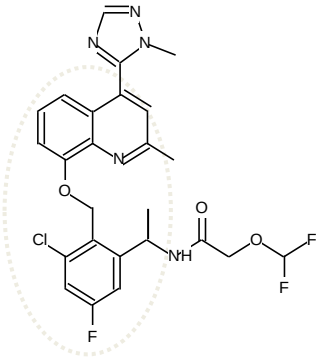




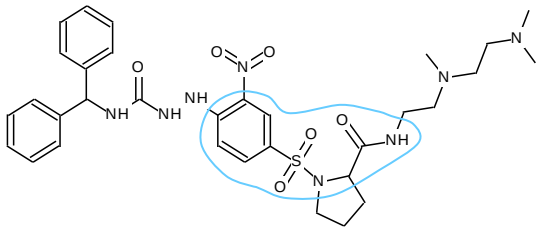
anatibant = LF16-0687



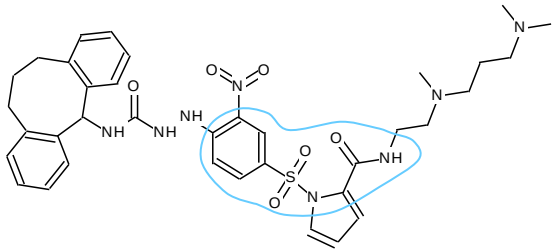
47a



Pharvaris
Compound 3

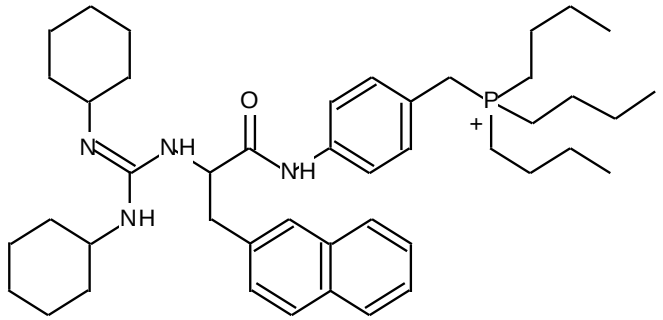


bradyzide



19c

WIN 64338



- First non-peptide BK B2R antagonist

Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 4693–4697, May 1994
Pharmacology

The nonpeptide WIN 64338 is a bradykinin B_2 receptor antagonist

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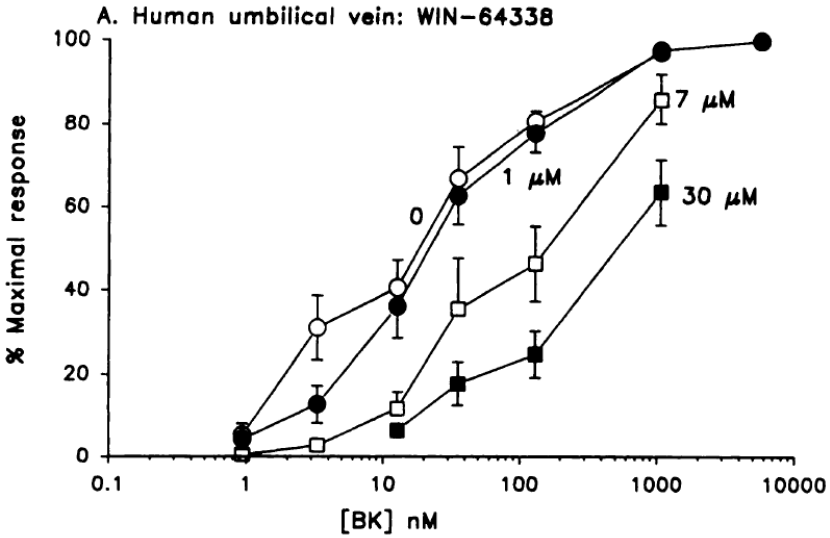
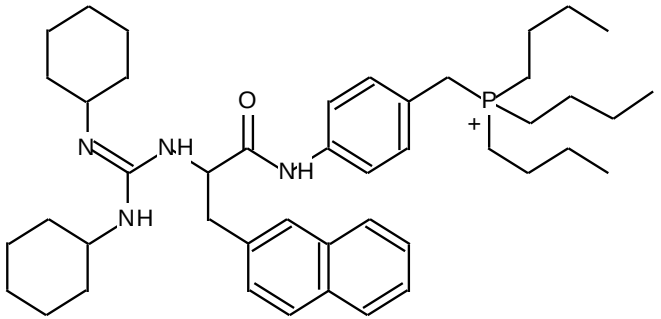
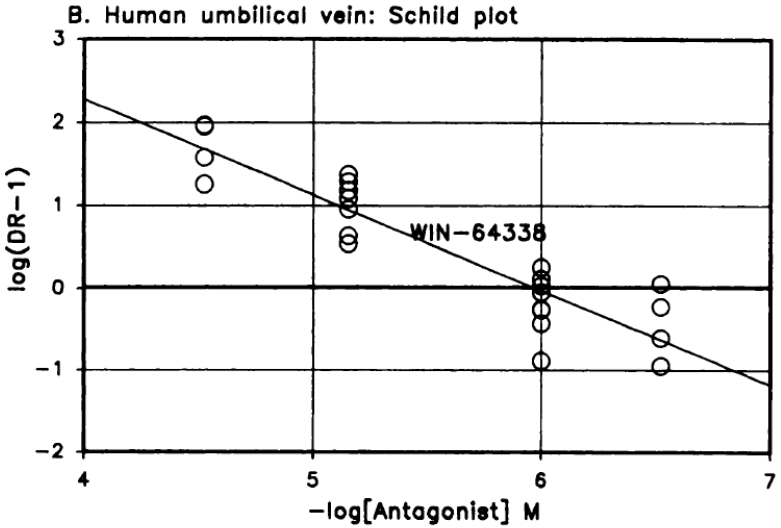


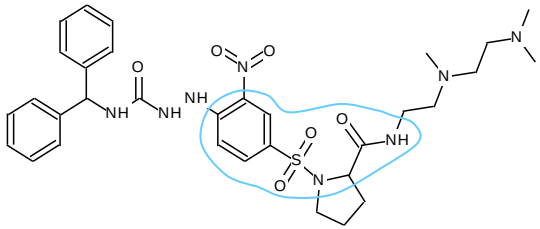




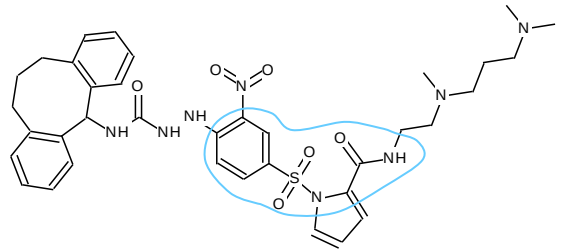
$pA_2 = 5.99$

Marceau et al. *J Pharmacol Exp Ther* 1994; **269**: 1136

The rodent-specific antagonist bradyzide is modified to improve potency at human B2R

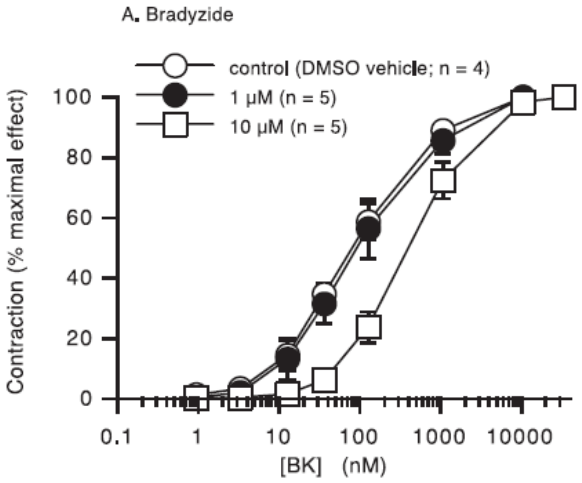


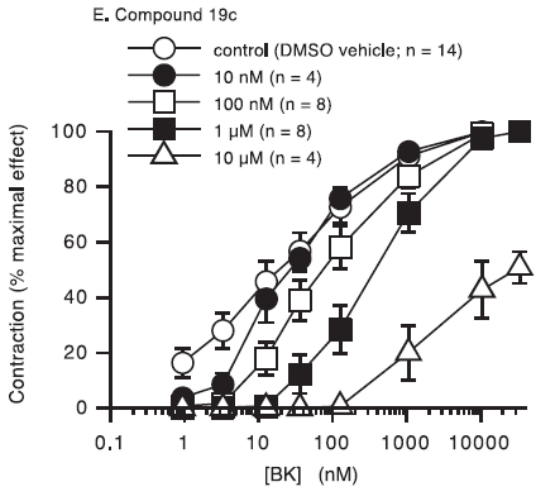
bradyzide

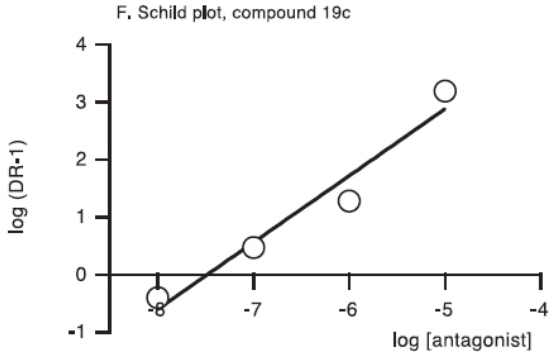


19c

$pA_2 = 7.53$

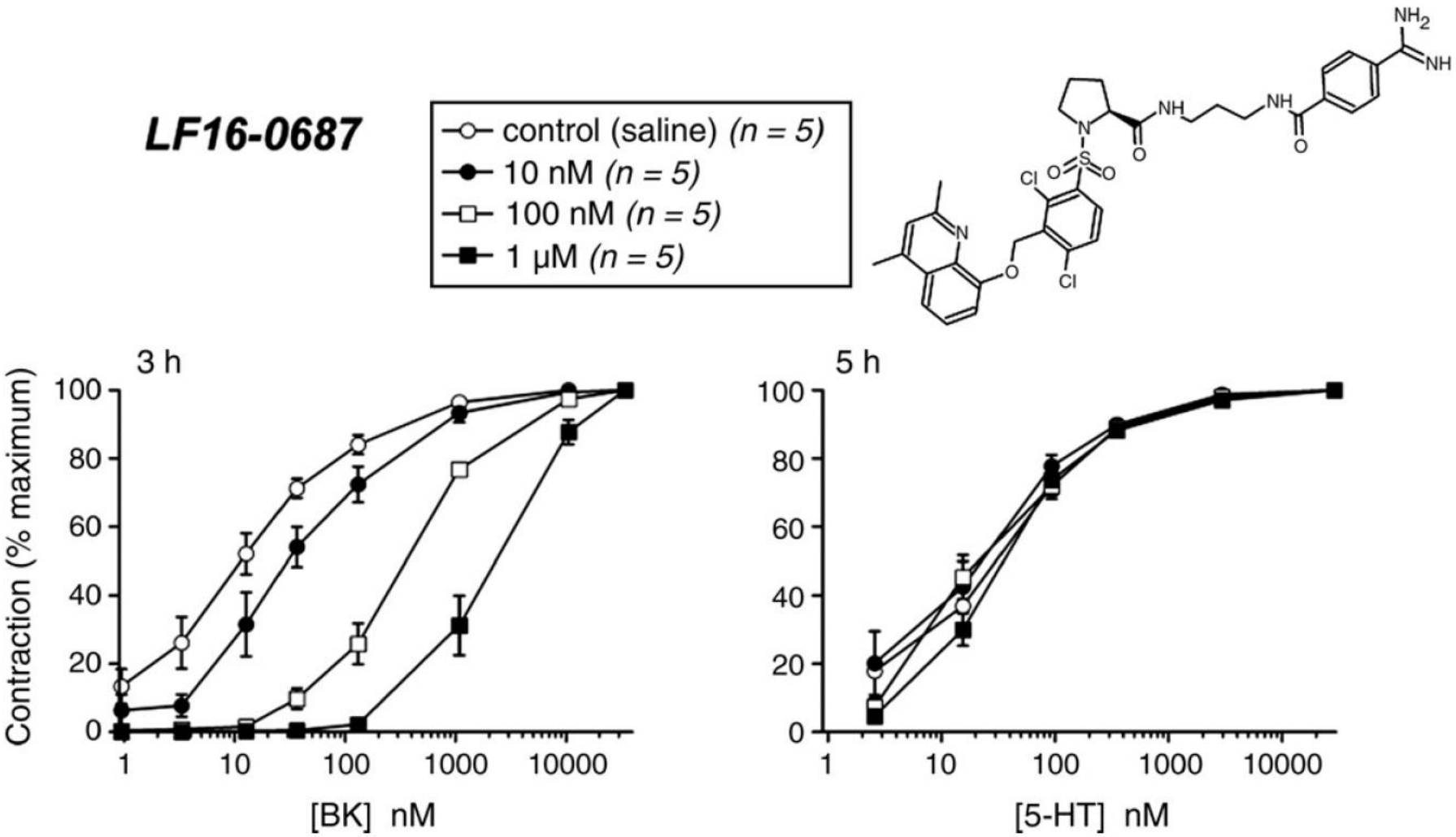






Marceau et al., *Int. Immunopharmacol.* 2003; **3**: 1529

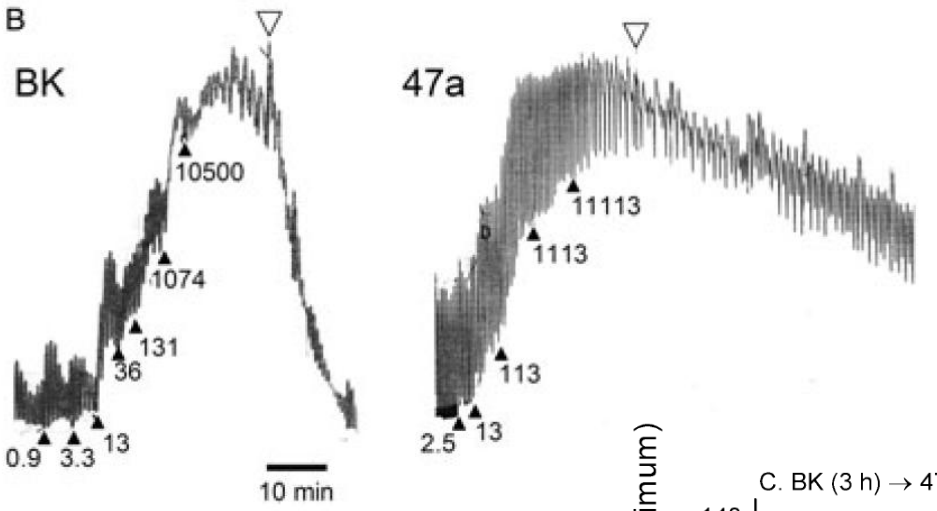
Anatibant (LF 16-0687)

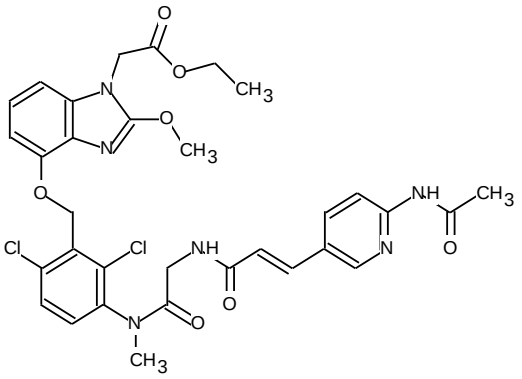


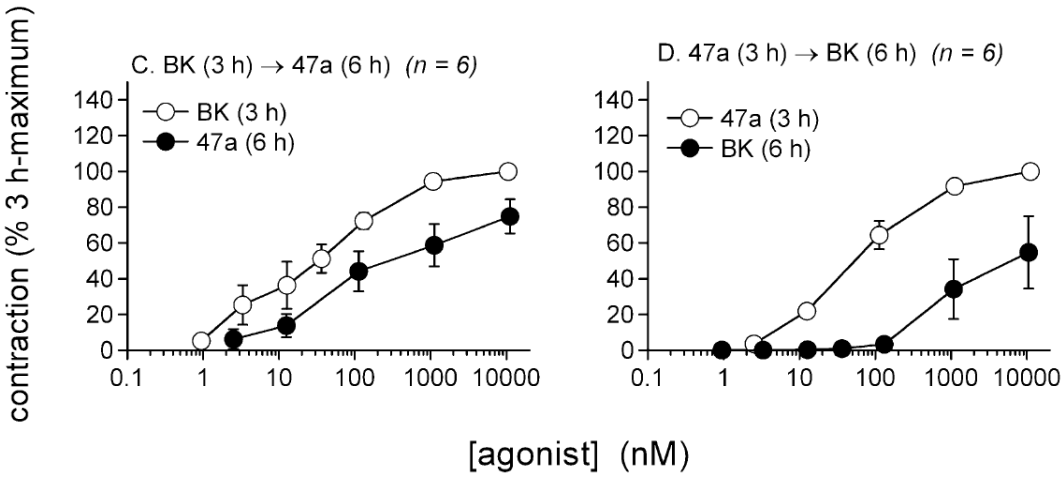
$pA_2 = 8.3$

Bawolak et al., *Regul.Peptides* 2007; **140**: 125

Compound 47a, a partial agonist identified by Fujisawa scientists

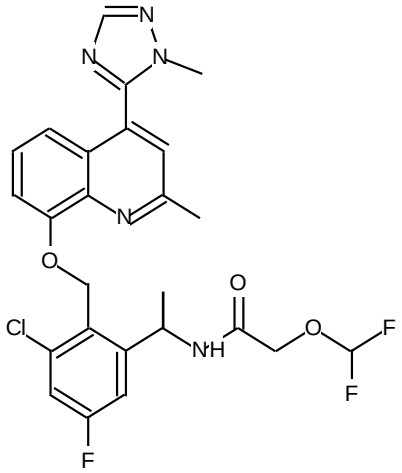


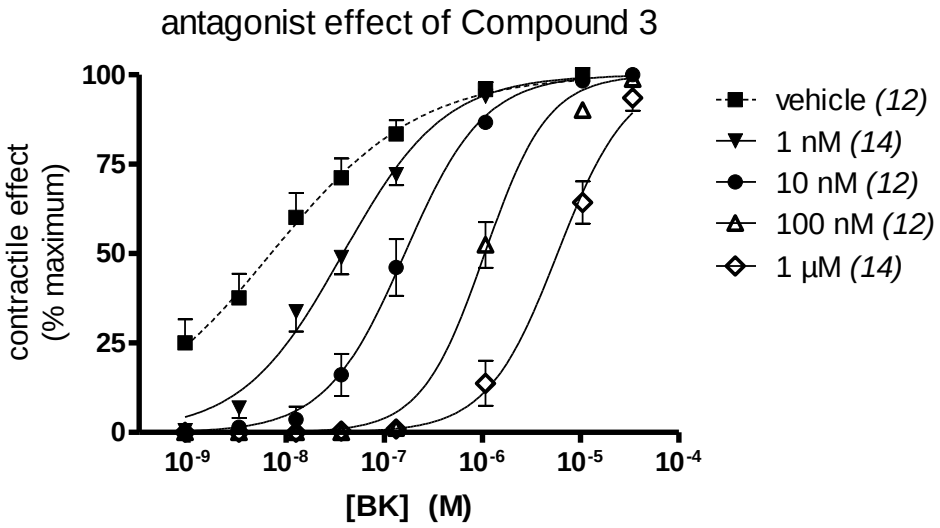


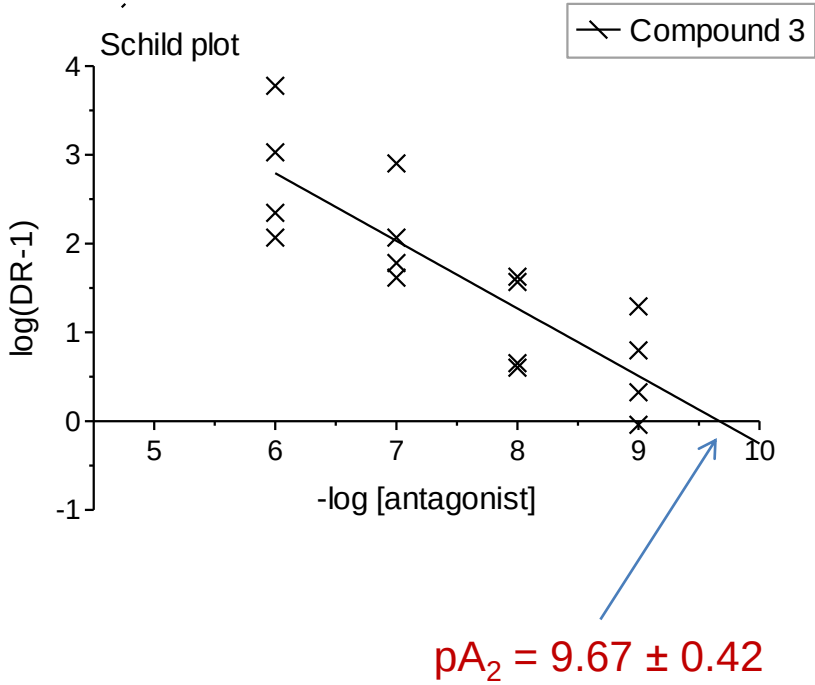


Bawolak et al., *Br. J. Pharmacol.* 2009; **158**: 1375

Pharvaris Compound 3



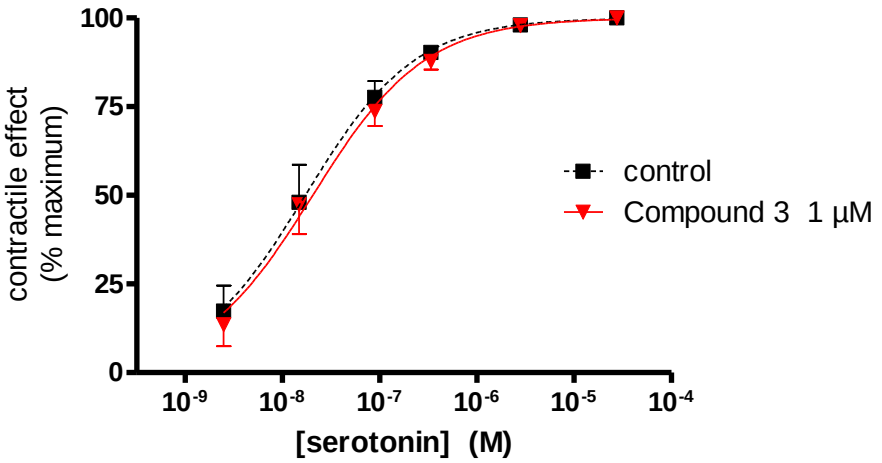




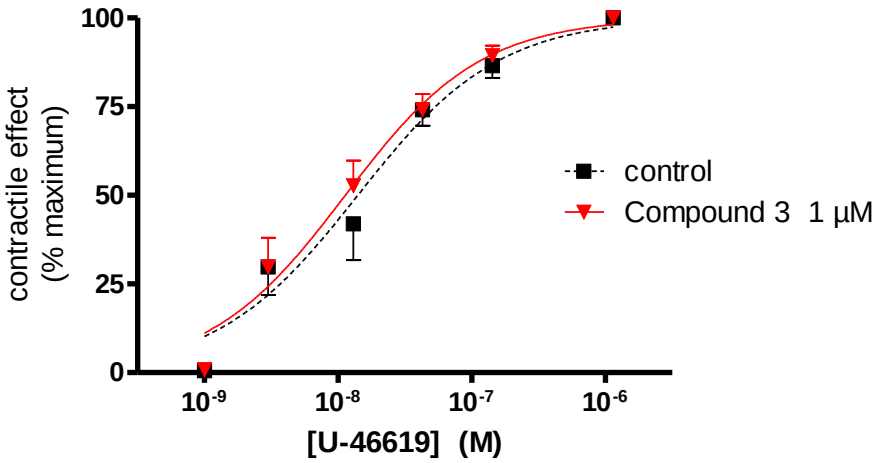
$pA_2 = 9.67 \pm 0.42$

Lesage et al., *Front. Pharmacol.* 2020; **11**: 916

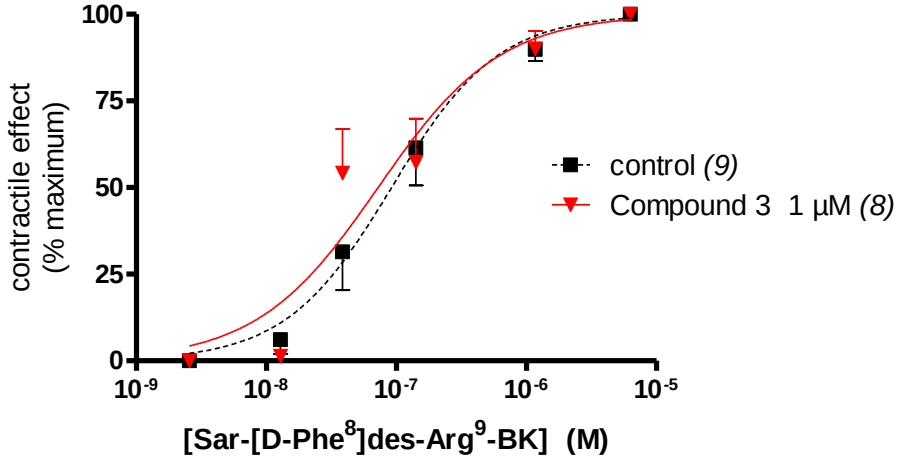
Compound 3 vs. serotonin
(n = 12)



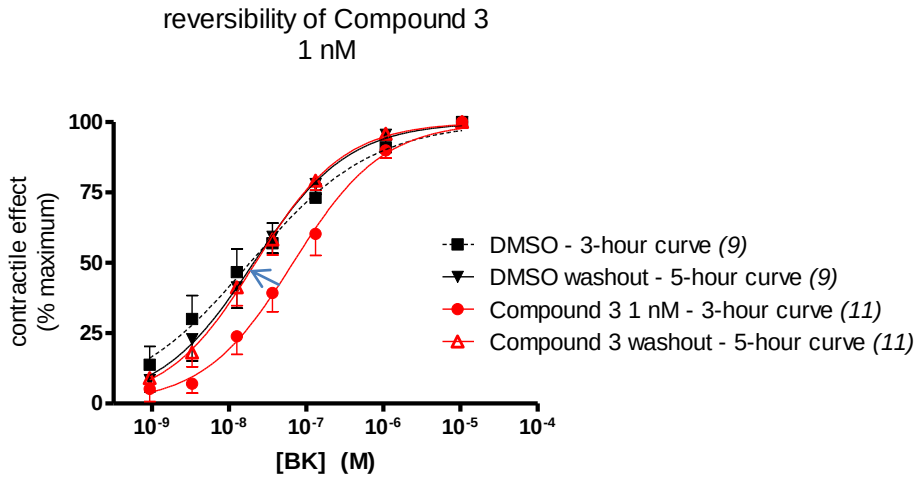
Coumpond 3 vs. U-46619
(n = 12)

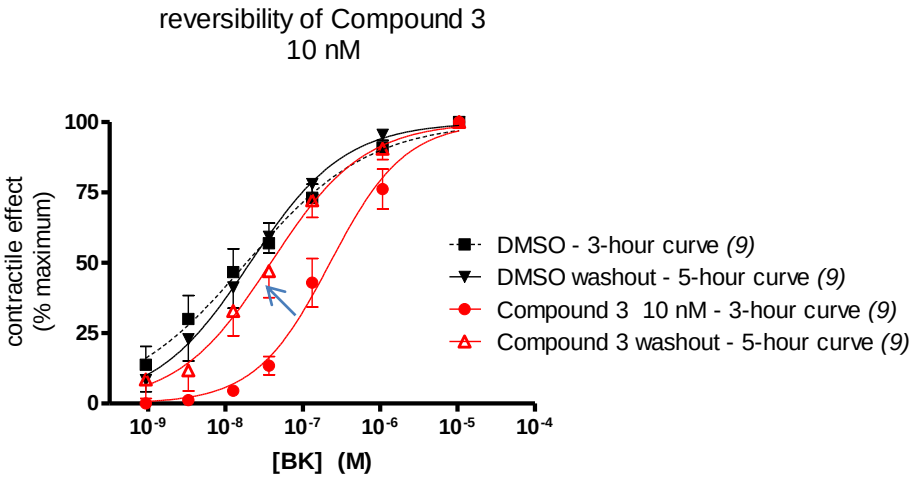


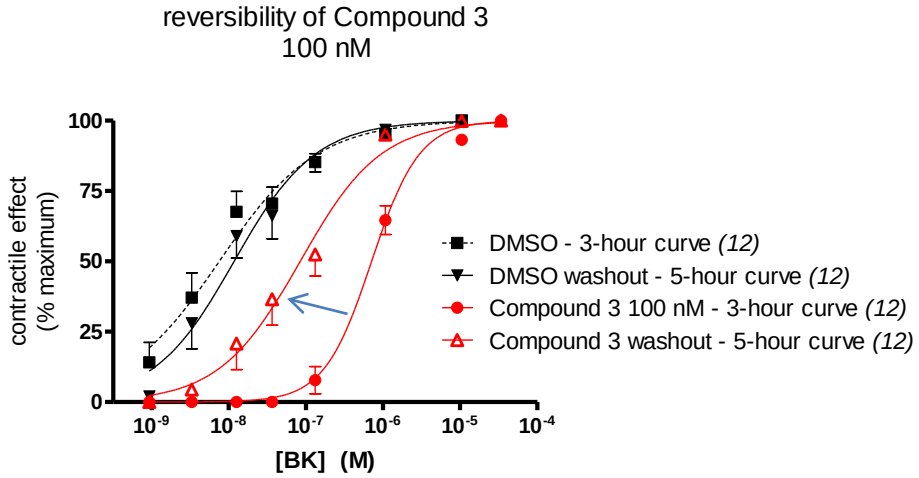
Compound 3 vs. Sar-[D-Phe^{8}]des-Arg^{9}-BK



Lesage et al., *Front. Pharmacol.* 2020; **11**: 916

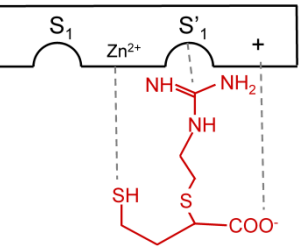






Lesage et al., *Front. Pharmacol.* 2020; **11**: 916

Verifying the properties of special peptide ligands, such as latent agonists activated by peptidases

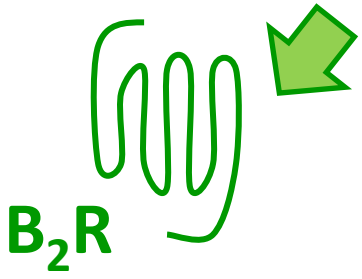


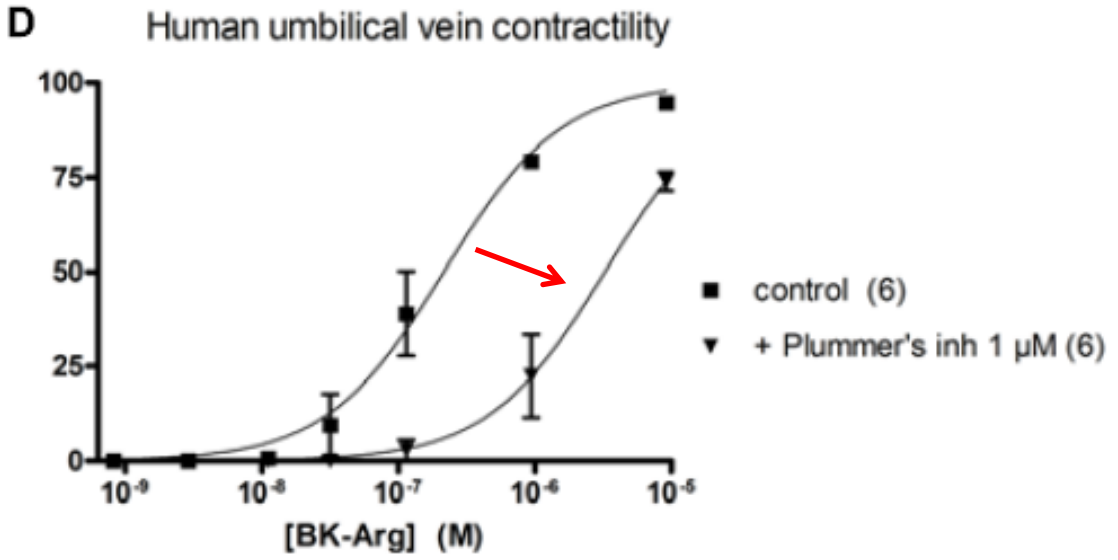
Plummer's inh.

Arg-carboxypeptidases

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)**



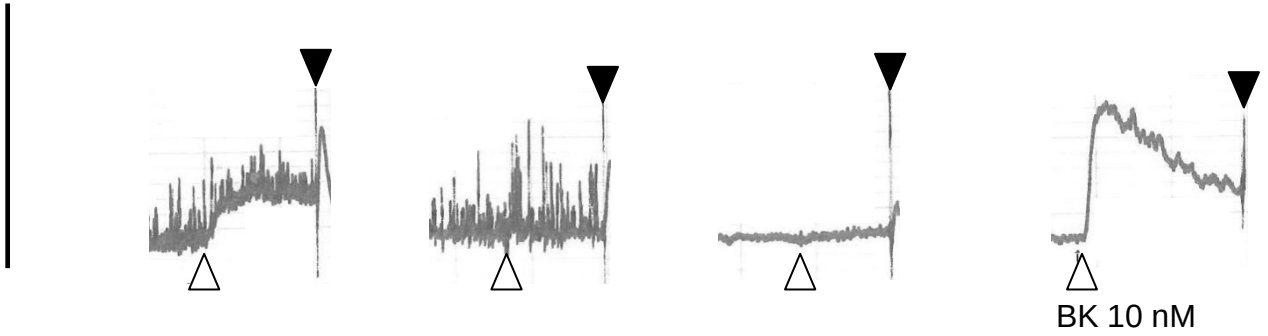


Charest-Morin et al., *Front. Pharmacol.* 2014; **5**: 32

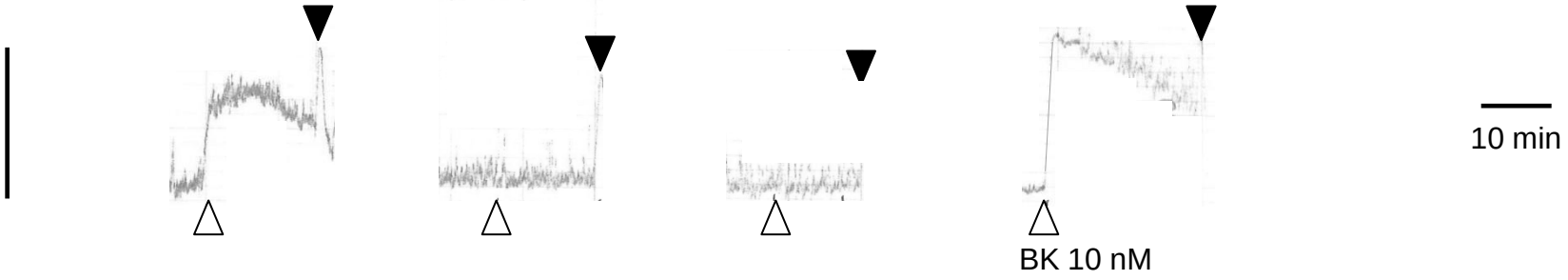
Verifying the claim that tissue kallikrein (KLK-1) is a direct BK B2R agonist

Tachyphylaxis, as well as the inhibitory effect of aprotinin or icatibant, indicated that KLK-1 releases a kinin from residual kininogen(s) adherent to the freshly isolated vein

KLK-1 1 nM at 1-hr intervals

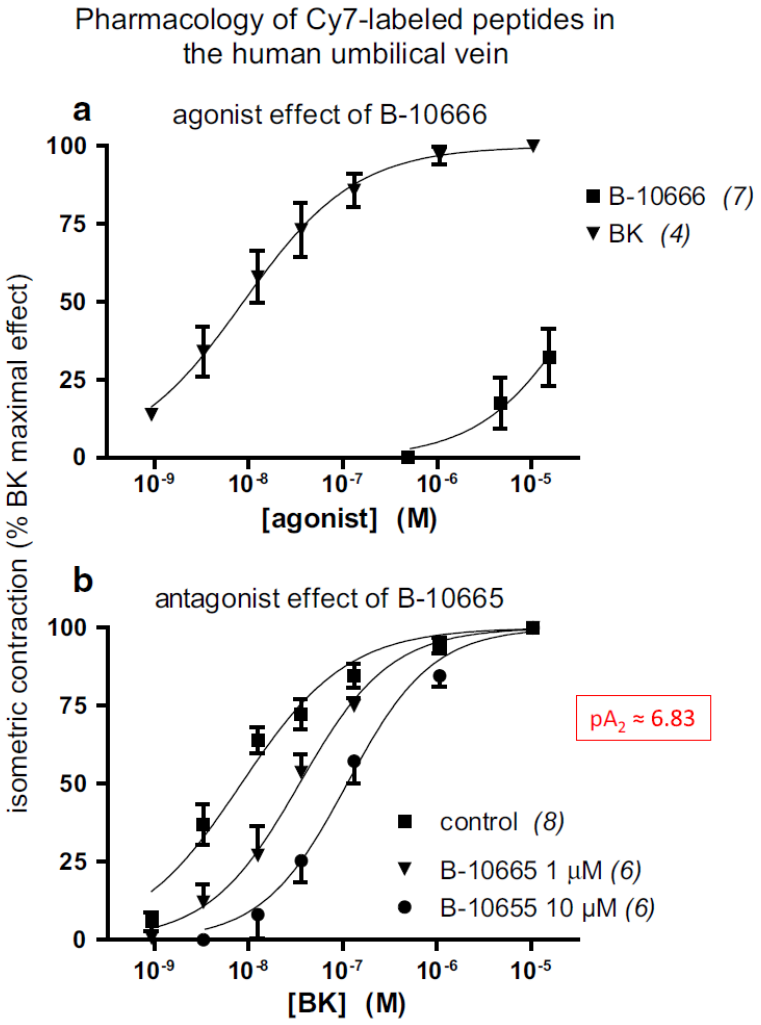


KLK-1 10 nM at 1-hr intervals

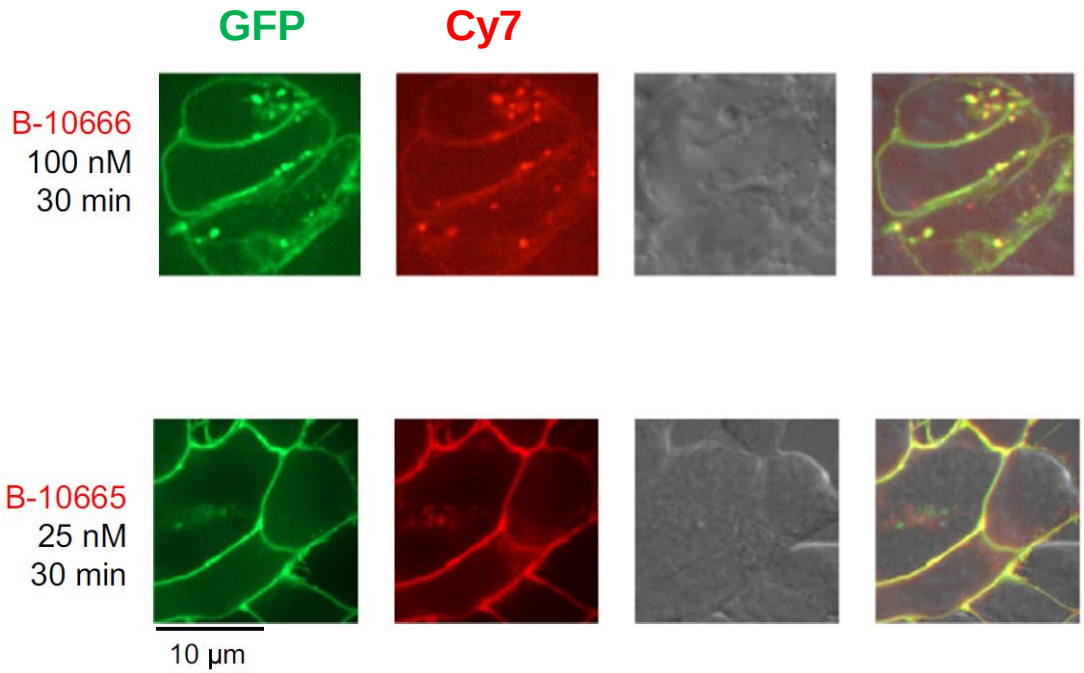


Jean et al., *Life Sci.* 2016; **155**: 180

Verifying the properties of special peptide ligands, such as fluorescent probes



HEK 293 cells expressing **B2R-GFP**



Gera et al., *BMC Res. Notes* 2016; **9**: 452

conclusions

- Antagonists have variable affinities for B2Rs from various mammalian species; the human umbilical vein smooth muscle naturally expresses the BK B2R

- Not a “low tech” approach: remarkably quantitative and complementary to molecular/cellular pharmacology. In a time scale of several hours, this assay allows determining potency, surmountability, residual agonist activity, specificity and reversibility

- This assay has an intermediate level of complexity between cellular and molecular pharmacology on one hand, and in vivo studies in subhuman primates on the other

funding

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

Fonds de recherche Santé Québec

DIAMEDICA INC

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