Angiotensin Type 1 Receptor and β-arrestin

Ki-Seok Kim, M.D.
Jeju National University Hospital

- GPCR in Heart
- RAS system
- Angiotensin II and AT1R
- β-arrestin
- Biased Agonism of AT1R
- AT1R β-arrestin biased Agonist
  - Effects on Cardiac Performance
  - Effects on HF model
  - Effects on Ischemia Reperfusion Injury

Classification of Receptors

- Intracellular receptors

  (for lipid soluble messengers)

  function in the nucleus as transcription factor

  to alter the rate of transcription of particular genes

- Plasma membrane receptors

  (for lipid insoluble messengers)

Types of Cell Membrane Receptor

1. G-Protein Coupled Receptor (GPCR)
2. Ion Channel Receptor
3. Tyrosine Kinase Receptor
4. Receptor with Intrinsic Enzymatic Activity

GPCR Classes

Class A: Rhodopsin like (most of mammalian GPCR)
Class B: Secretin like
Class C: Metabotropic glutamate / pheromone
Class D: Fungal pheromone
Class E: cAMP receptors

Frizzled/Smoothened family

Putative families:
* Ocular albinism proteins
* Insect odorant receptors
* Plant Mlo receptors
* Nematode chemoreceptors
* Vomeronasal receptors (V1R & V3R)
* Taste receptors T2R

Orphans:
* Putative / unclassified GPCRs

non-GPCR families:
* Class Z: Archaeal/bacterial/fungal opsins

GPCR Classes

* Class A Rhodopsin like

- o Amine
- **o Peptide**
- o Hormone protein
- o (Rhod)opsin
- o Olfactory
- o Prostanoid
- o Nucleotide-like
- o Cannabinoid
- o Platelet activating factor
- o Gonadotropin-releasing hormone
- o Thyrotropin-releasing hormone & Secretagogue
- o Melatonin
- o Viral
- o Lysosphingolipid & LPA (EDG)
- o Leukotriene B4 receptor
- o Class A Orphan/other

* Class B Secretin like

- o Calcitonin
- o Corticotropin releasing factor
- o Gastric inhibitory peptide
- o Glucagon
- o Growth hormone-releasing hormone
- o Parathyroid hormone
- o PACAP
- o Secretin
- o Vasoactive intestinal polypeptide
- o Diuretic hormone
- o EMR1
- o Latrophilin
- o Brain-specific angiogenesis inhibitor (BAI)
- o Methuselah-like proteins (MTH)
- o Cadherin EGF LAG (CELSR)
- o Very large G-protein coupled receptor

* Class C Metabotropic glutamate / pheromone

- o Metabotropic glutamate
- o Calcium-sensing like
- o Putative pheromone receptors
- o GABA-B
- o Orphan GPRC5
- o Orphan GPCR6
- o Bride of sevenless proteins (BOSS)
- o Taste receptors (T1R)

* Class D Fungal pheromone

- o Fungal pheromone A-Factor like (STE2,STE3)
- o Fungal pheromone B like (BAR,BBR,RCB,PRA)
- o Fungal pheromone M- and P-Factor

* Class E cAMP receptors

* Frizzled/Smoothened family
  - o frizzled
  - o Smoothened

Putative families:

- * Ocular albinism proteins
- * Insect odorant receptors
- * Plant Mlo receptors
- * Nematode chemoreceptors
- * Vomeronasal receptors (V1R & V3R)
- * Taste receptors T2R

Orphans:

- * Putative / unclassified GPCRs

non-GPCR families:

- * Class Z Archaeal/bacterial/fungal opsins

GPCR Ligands

Rhodopsin family: amine receptors
- Acetylcholine (muscarinic)
- Adrenaline
- Dopamine
- Histamine
- Serotonin
- Octopamine

Rhodopsin family: peptide receptors
Angiotensin
- Apelin
- Bombesin
- Bradykinin
- C5a anaphylatoxin
- CC Chemokine
- CXC Chemokine
- CX3C Chemokine
- C Chemokine
- Cholecystokinin
- Endothelin
- fMet-Leu-Phe
- Galanin
- Ghrelin
- KiSS1-derived peptide
- Melanocortin
- Motilin
- Neuromedin U
- Neuropeptide FF
- Neuropeptide S
- Neuropeptide Y
- Neuropeptide W / neuropeptide B
- Neurotensin
- Orexigenic neuropeptide QRFP
- Opioid
- Orexin
- Oxytocin
- Prokineticin
- Somatostatin
- Tachykinin
- Urotensin II
- Vasopressin
- Protease-activated (thrombin)
- Adrenomedullin (G10D)
- GPR37 / endothelin B like
- Chemokine receptor like
- Melanin-concentrating hormone
- Follicle stimulating hormone
- Lutropin-choriogonadotropic hormone
- Thyrotropin

Rhodopsin family: other receptors
- Rhodopsin
- Olfactory
- Prostaglandin
- Prostacyclin
- Thromboxane
- Adenosine
- Purine / pyrimidine
- Cannabinoid
- Platelet activating factor
- Gonadotropin-releasing hormone
- Thyrotropin-releasing hormone
- Melatonin
- Lysosphingolipid and LPA (EDG)
- Leukotriene B4 receptor
- SREB
- Mas proto-oncogene & Mas-related (MRGs)
- RDC1
- EBV-induced
- Relaxin
- LGR like
- Free fatty acid
- G protein-coupled bile acid
- Nicotinic acid
- GPR
- GPR45 like
- Cysteinyl leukotriene
- Putative / unclassified Class A GPCRs

Secretin family
- Calcitonin
- Corticotropin releasing factor
- Gastric inhibitory peptide
- Glucagon
- Growth hormone-releasing hormone
- Parathyroid hormone
- PACAP
- Secretin
- Vasoactive intestinal polypeptide
- EMR1
- Latrophilin
- Brain-specific angiogenesis inhibitor (BAI)
- Methuselah-like proteins (MTH)
- Cadherin EGF LAG (CELSR)
- Putative / unclassified Class B GPCRs

Metabotropic glutamate family
- Glutamate (metabotropic)
- Extracellular calcium-sensing
- GABA-B
- Pheromone (V2R)
- Taste receptors (T1R)
- Orphan GPRC5
- Orphan GPCR6
- Bride of sevenless proteins (BOSS)
- Putative / unclassified Class C GPCRs

Other families
- Frizzled / Smoothened family
- Ocular albinism proteins
- Vomeronasal receptors (V1R)
- Taste receptors (T2R)
- Insect odorant receptors
- Nematode chemoreceptors
- Plant Mlo receptors
- Fungal pheromone
- cAMP (Dictyostelium)
- Bacterial rhodopsin

GPCR in Heart

- Adrenergic receptor
- Cholinergic receptor
- Adenosine receptor
- Endothelin receptor
- Angiotensin receptor
  - Class A: Rhodopsin like – Peptide Receptor
  - Cardiomyocyte, Fibroblast, VSMC…

Why is GPCR important?

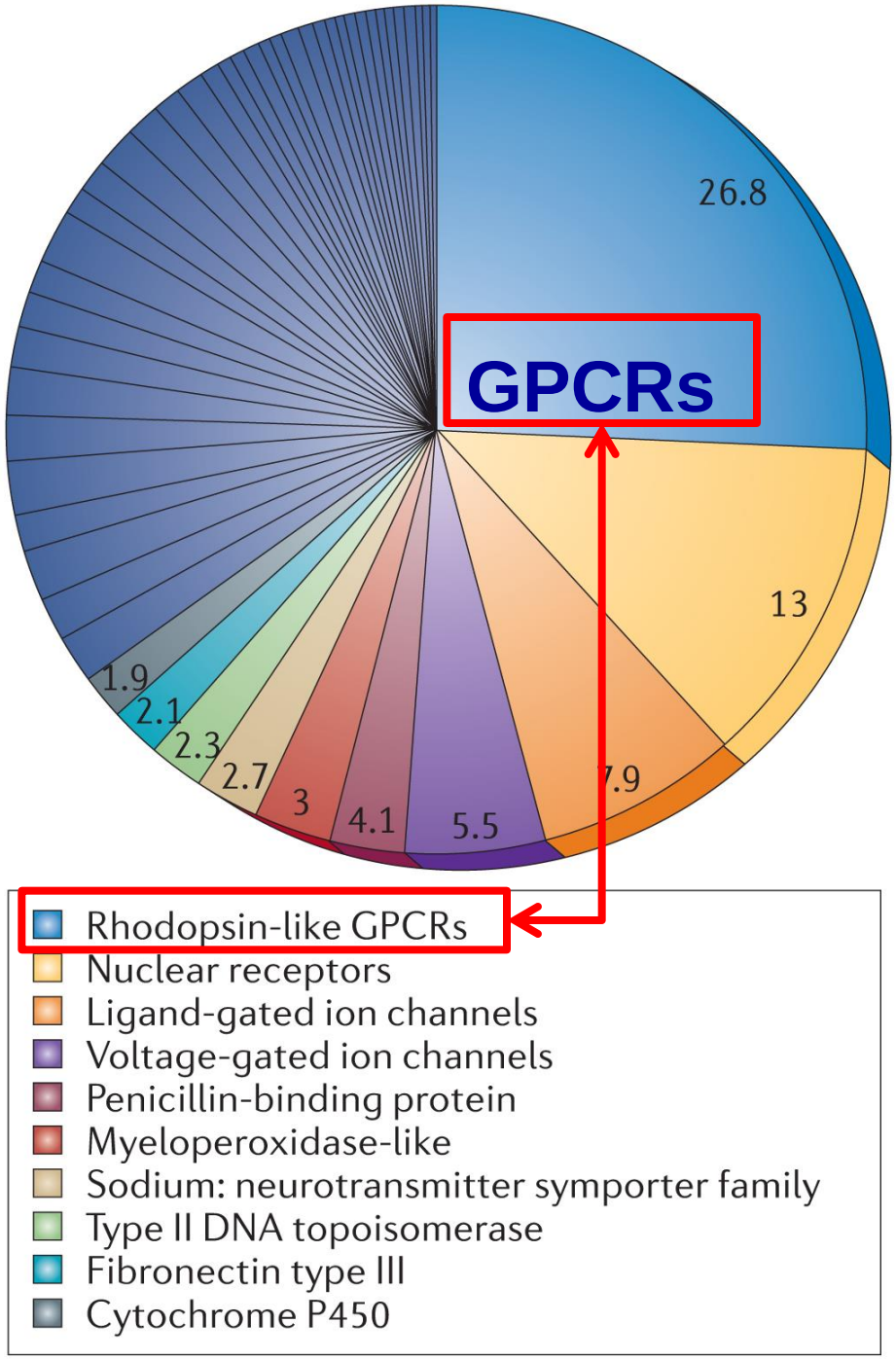
- GPCRs in disease states

  Disease states associated with GPCR mutations

- GPCRs are good drug targets

  50% of subscription drugs interact with GPCRs

  Hypertension, Stomach ulcers, Migraine, Allergies



GPCRs are the largest single target class for drug action

Overington et al.
Nature Reviews Drug Discovery, 2006

nature REVIEWS DRUG DISCOVERY

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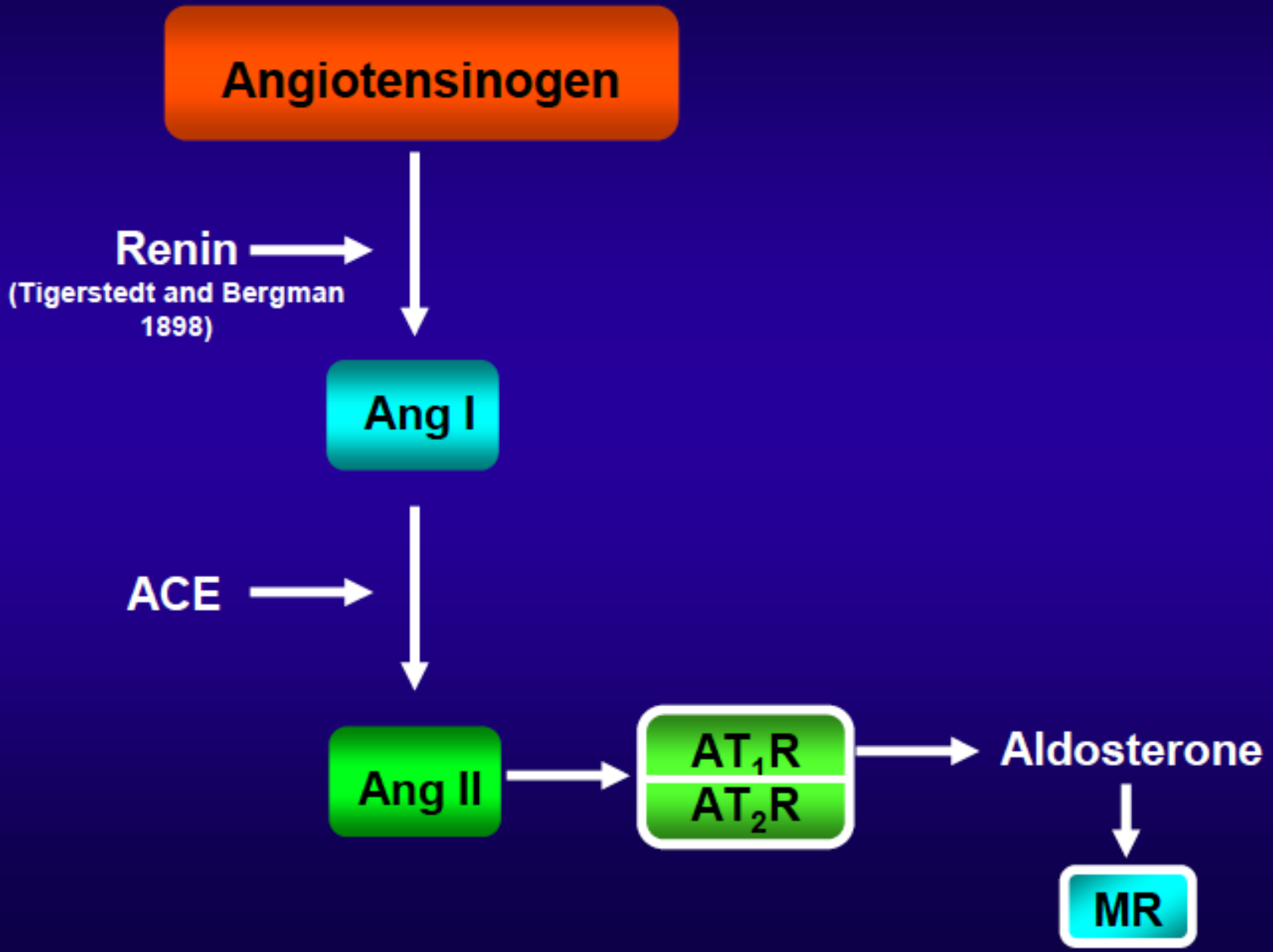
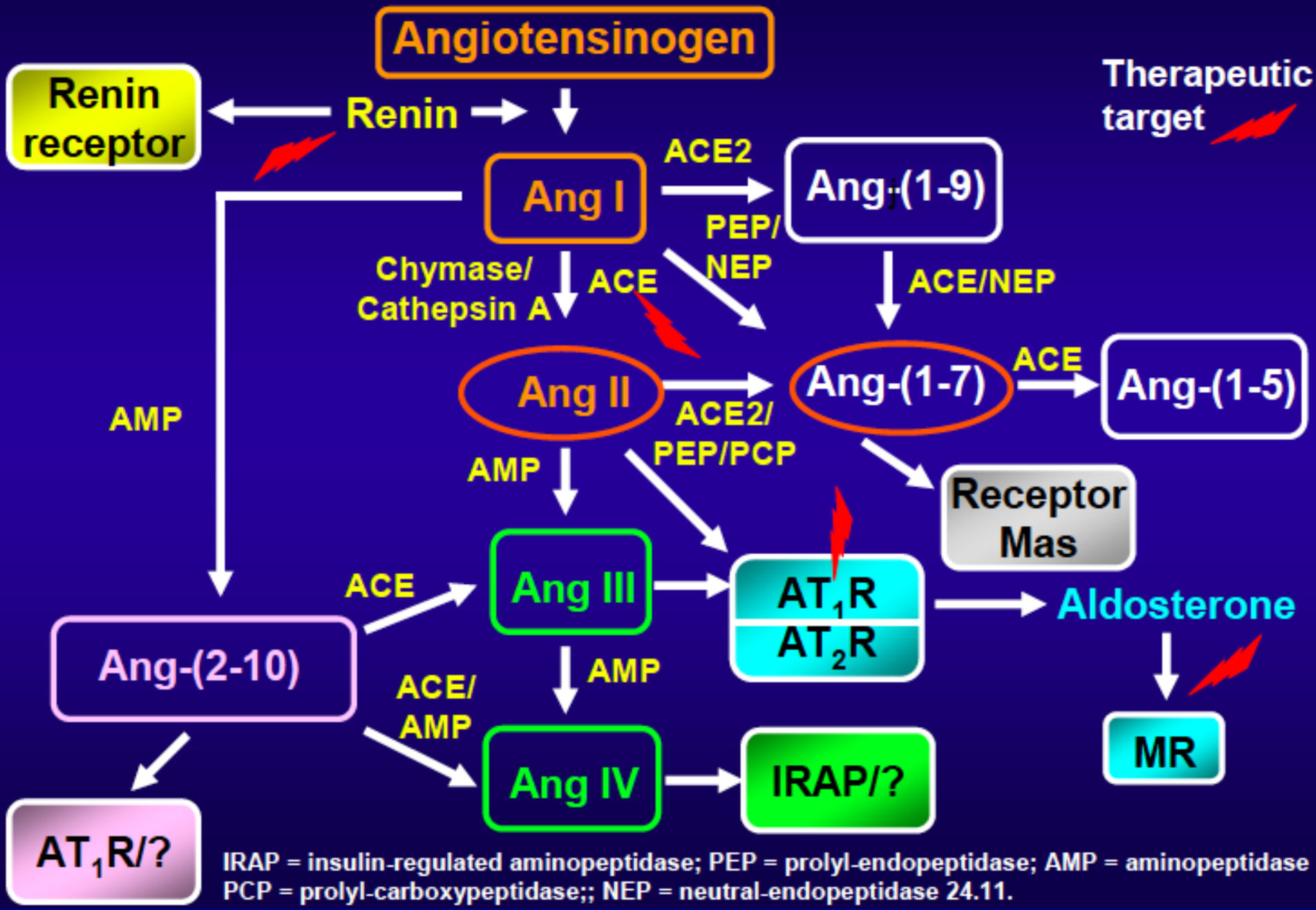
The RAS – a Simple Paradigm



The RAS – a Complex Network



IRAP = insulin-regulated aminopeptidase; PEP = prolyl-endopeptidase; AMP = aminopeptidase
PCP = prolyl-carboxypeptidase;; NEP = neutral-endopeptidase 24.11.

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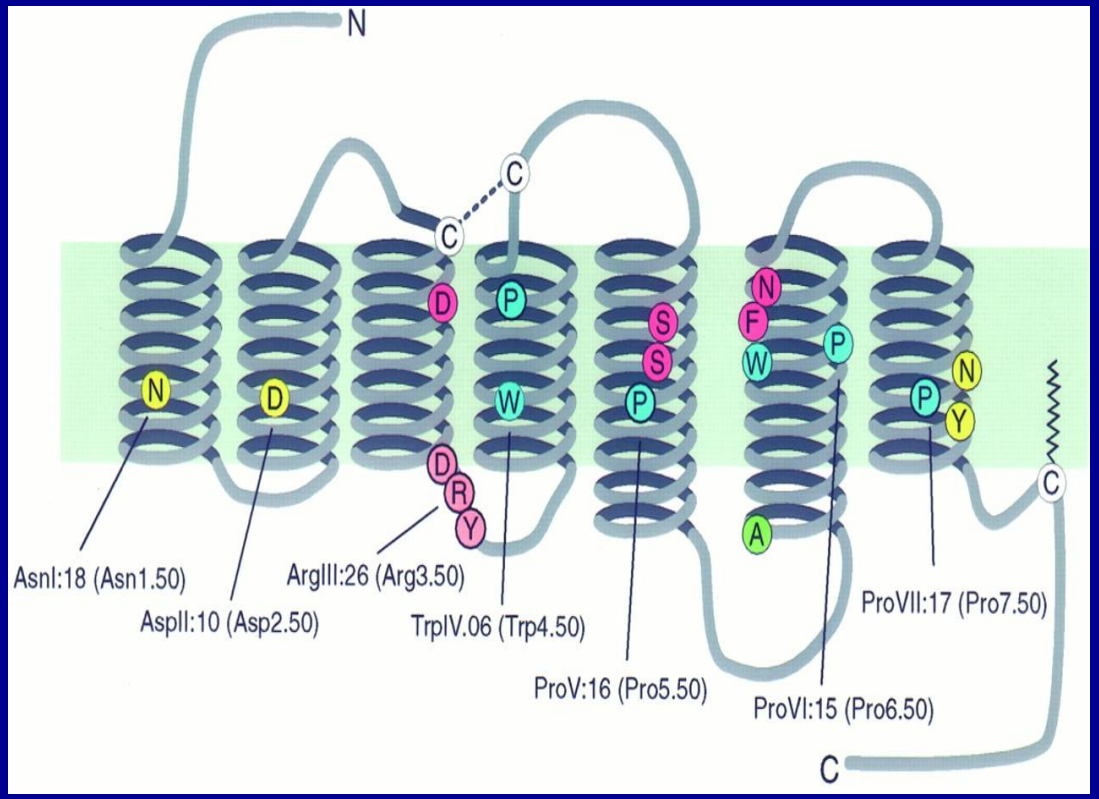
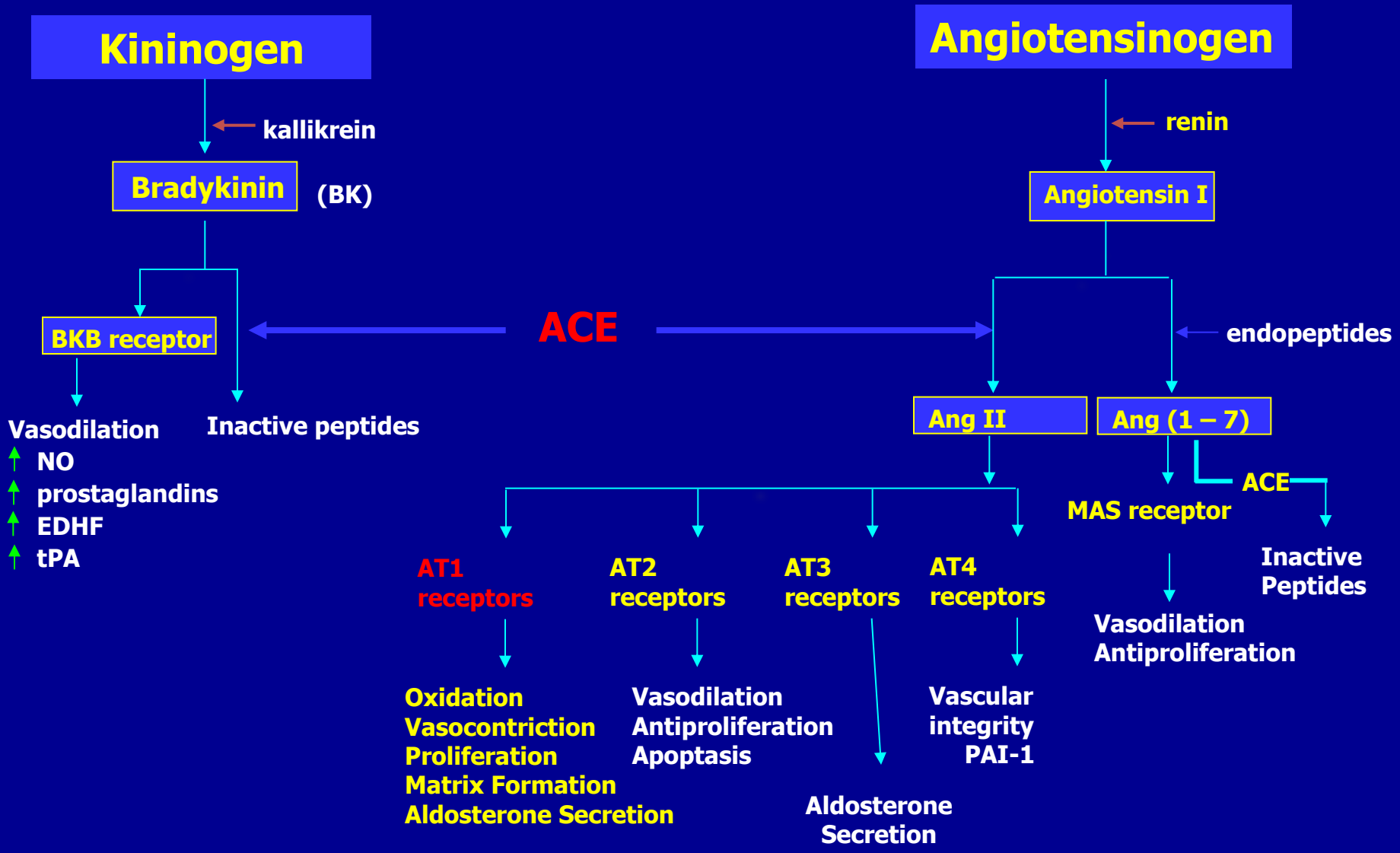
Angiotensin Type 1 Receptor

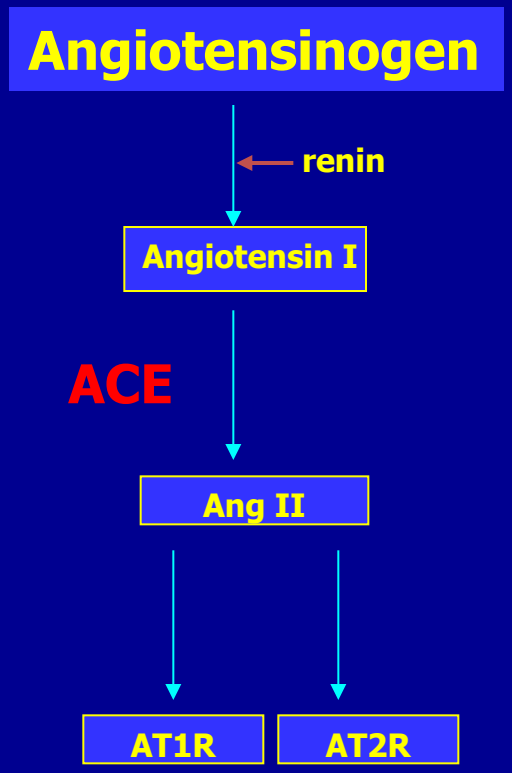


- 7 TMR ; AKA GPCR
- N-termial
  ; ligand binding site
- C-terminal
  ; GDP binding site

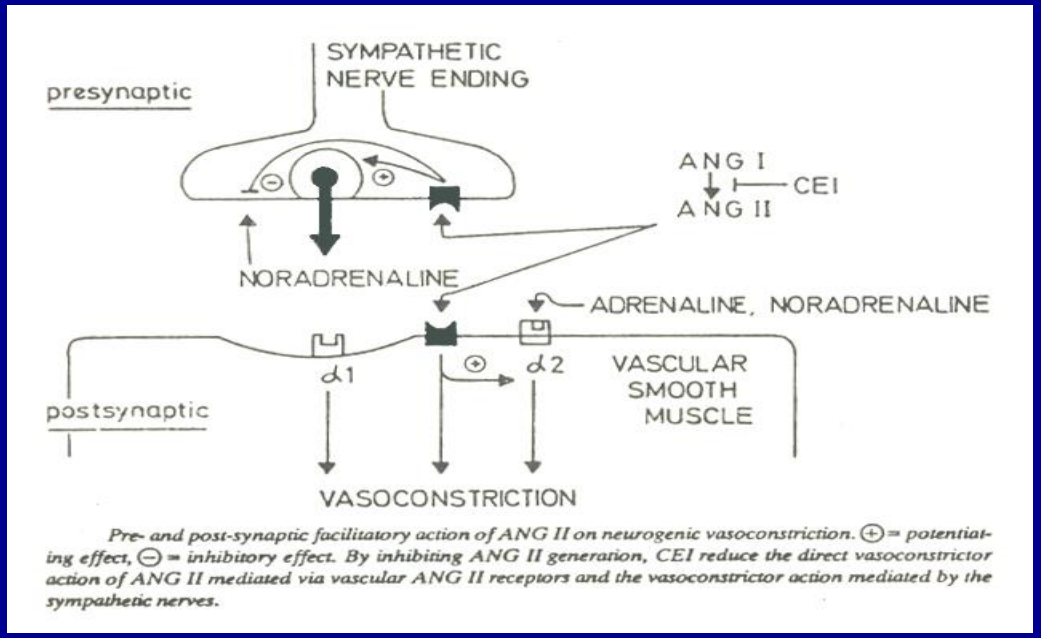
Components and Major Actions of the Kallikrein-Kinin and Renin-Angiotensin systems



Angiotensin II & AT1R

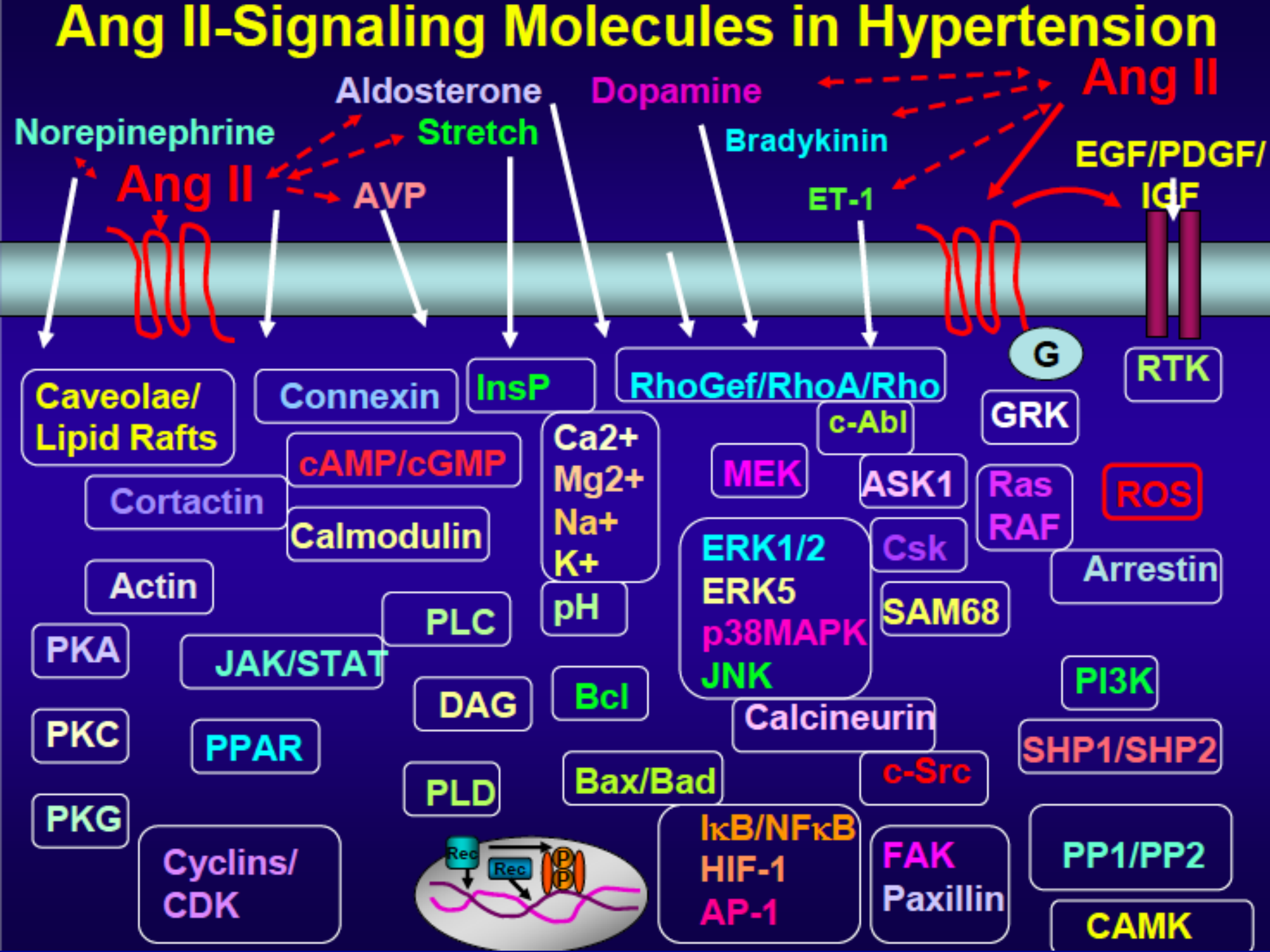


- Octapeptide hormone
- most potent presser substance known (direct arter iolar vasoconstriction)
- increases sympathetic vasomotor discharge
- decreases renin release (negative feedback)



Pre- and post-synaptic facilitatory action of ANG II on neurogenic vasoconstriction. ⊕ = potentiating effect, ⊖ = inhibitory effect. By inhibiting ANG II generation, CEI reduce the direct vasoconstrictor action of ANG II mediated via vascular ANG II receptors and the vasoconstrictor action mediated by the sympathetic nerves.

Ang II-Signaling Molecules in Hypertension



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Discovery of β-arrestin

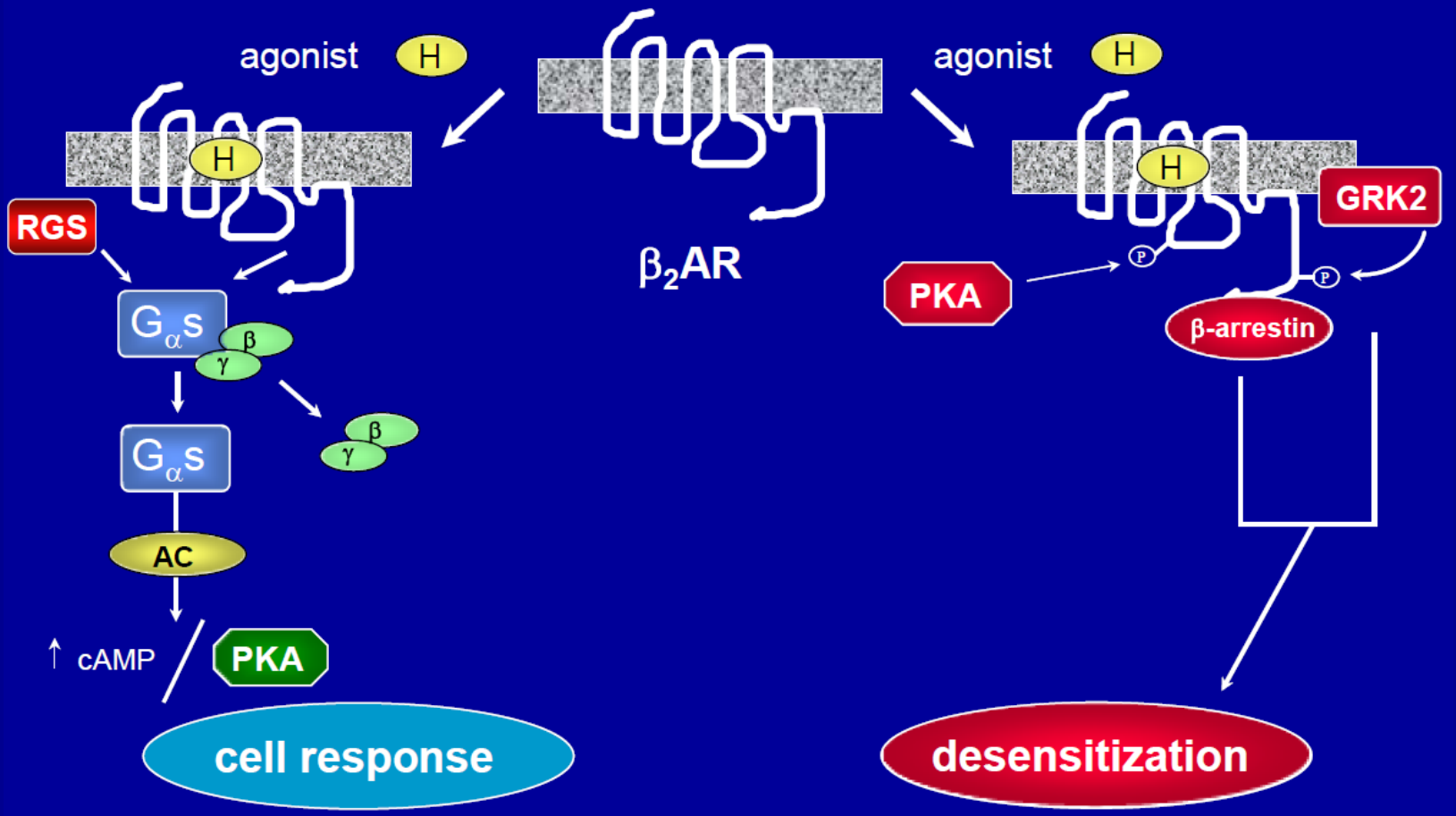
- Purified βARK (GRK2) loses ability to desensitize isolated β2-AR (Benovic et al '85,'86)
- Abundant retinal protein, "48 K protein" or "S Antigen" works with rhodopsin kinase to deactivate rhodopsin renamed arrestin (Kuhn, et al '87)
- S antigen (48 kDa protein) cloned (Shinohara et al '87)
- β-arrestin1 cloned – (Lohse et al '90)
- β-arrestin2 cloned – (Attramadal et al '92)

Arrestins

	AKA	Distribution	7MSR
Arrestin 1	(Visual Arrestin)	Retinal rods	Rhodopsin
β-Arrestin 1	(Arrestin 2)	Ubiquitous	Most
β-Arrestin 2	(Arrestin 3)	Ubiquitous	Most
X Arrestin	(Arrestin 4)	Retinal cones	Opsins

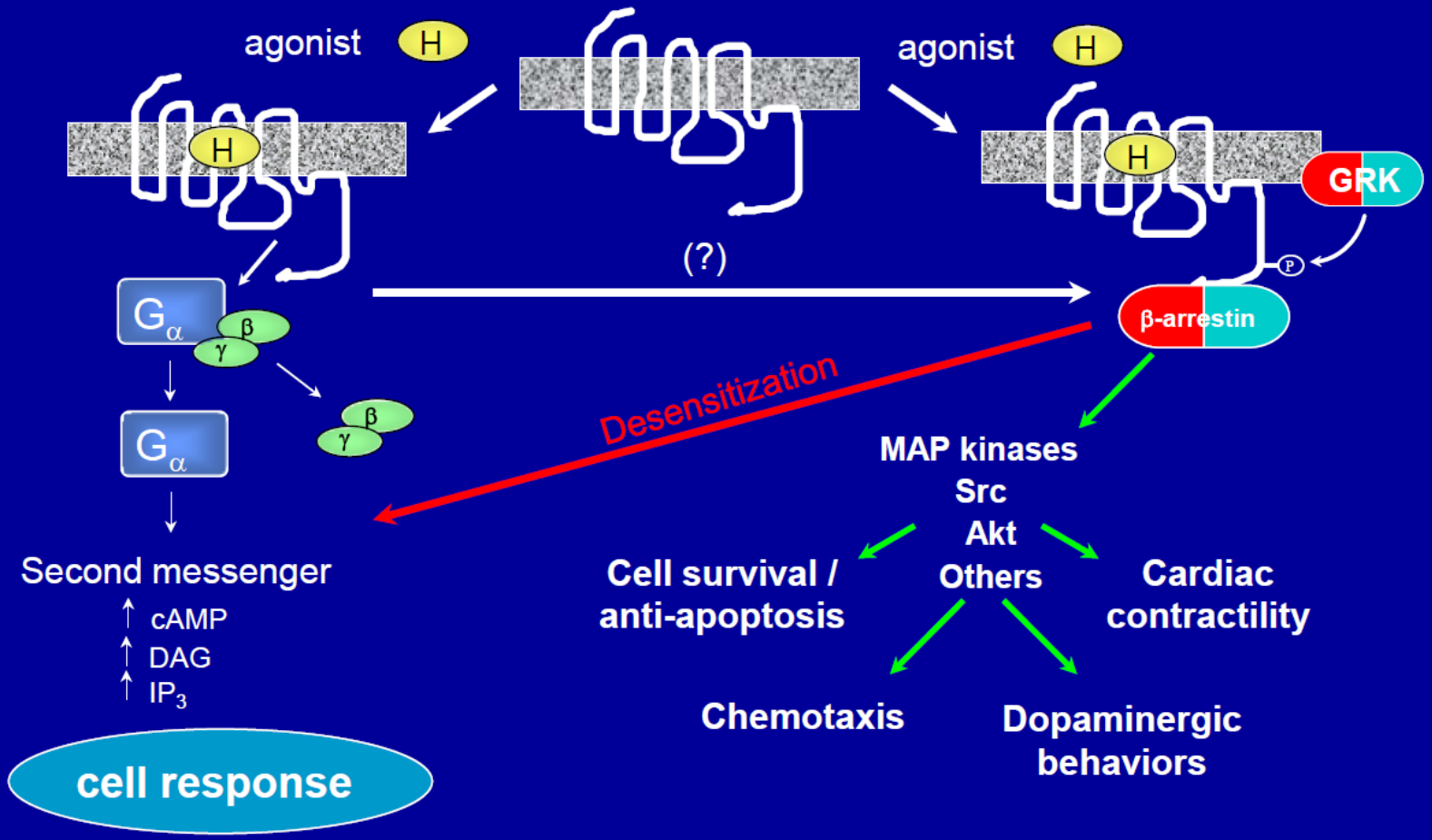
Sigler PB & Schubert C, 2001 Structure, Vol. 9, 869–880.

Role of β-arrestin
Two Paradigms: Activation & Desensitization



Adapted from Lefkowitz 2012 Nobel prize Lecture

Role of β-arrestin
New Signaling Paradigm



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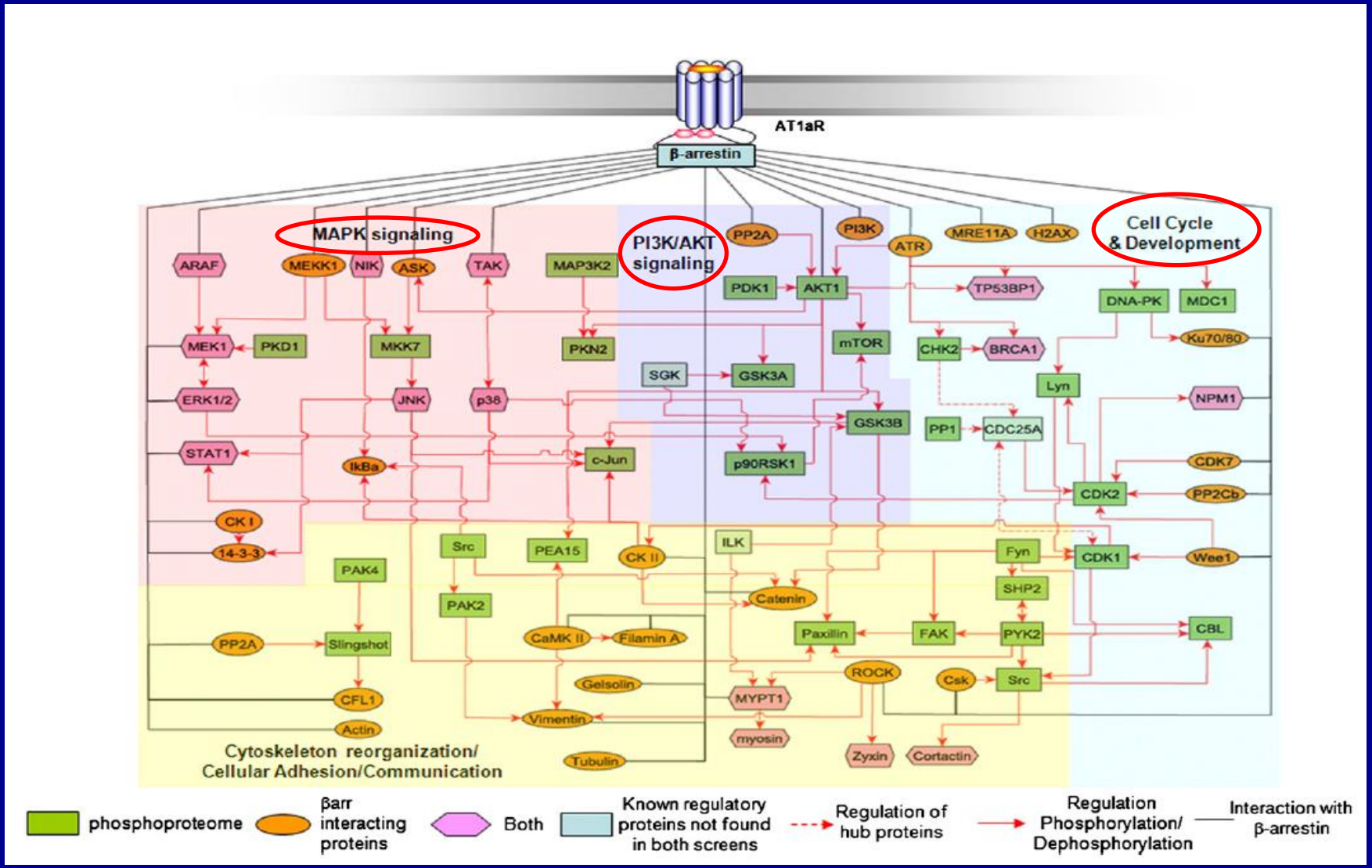
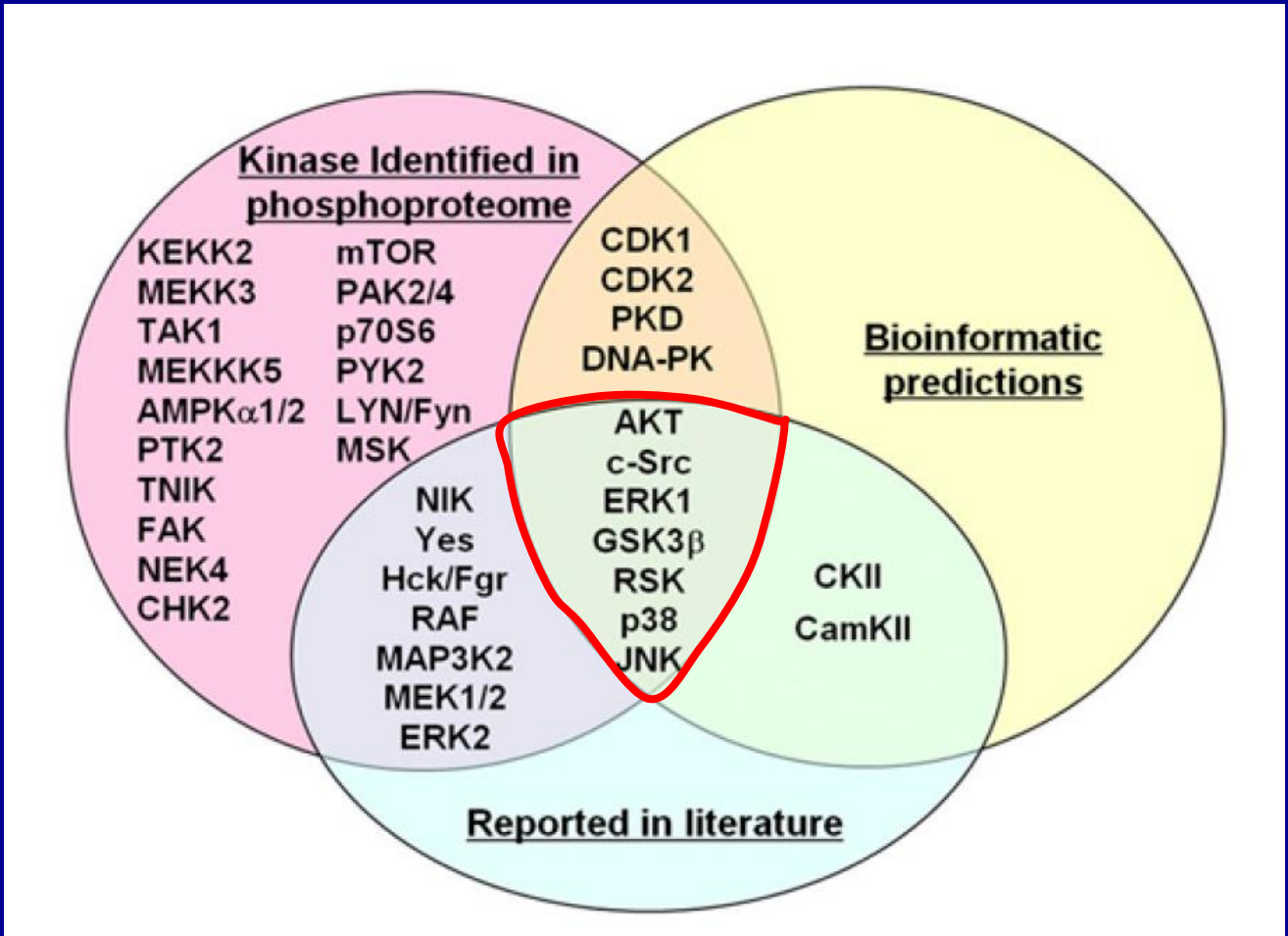
β-arrestin Signaling of AT1R



Xiao et al PNAS 2010

Protein kinases involved in β-arrestin–mediated cellular signaling downstream of AT1aR



Xiao et al PNAS 2010

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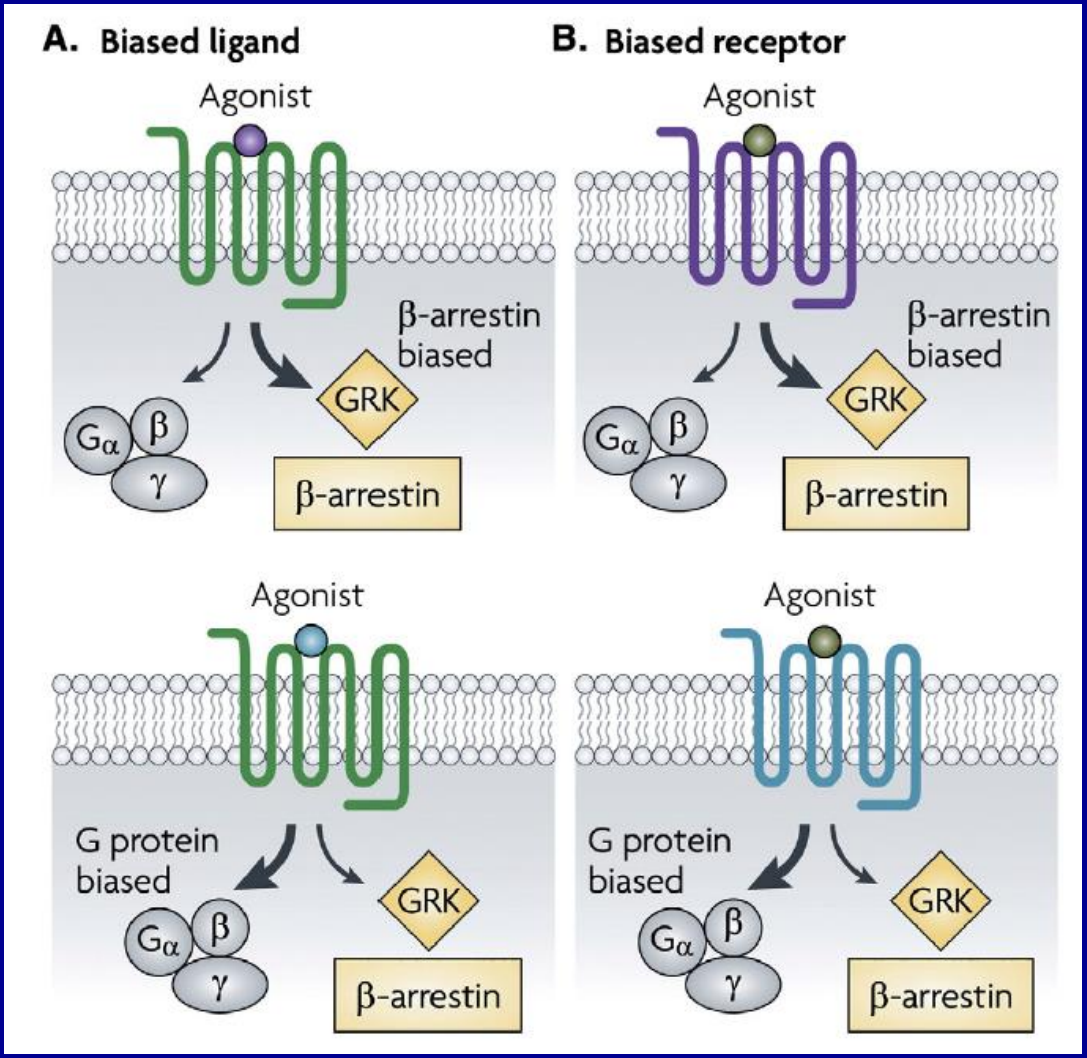
Biased ligands and biased receptors

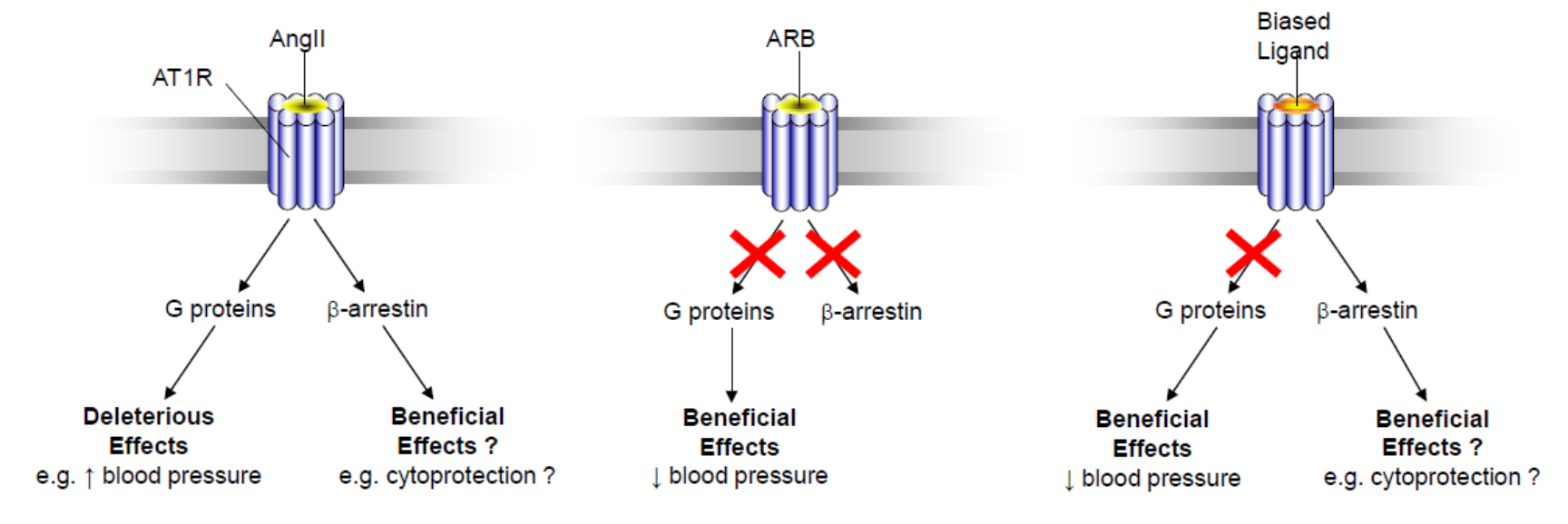


N. Noor et al Journal of Molecular and Cellular Cardiology 2011

β-arrestin biased agonist

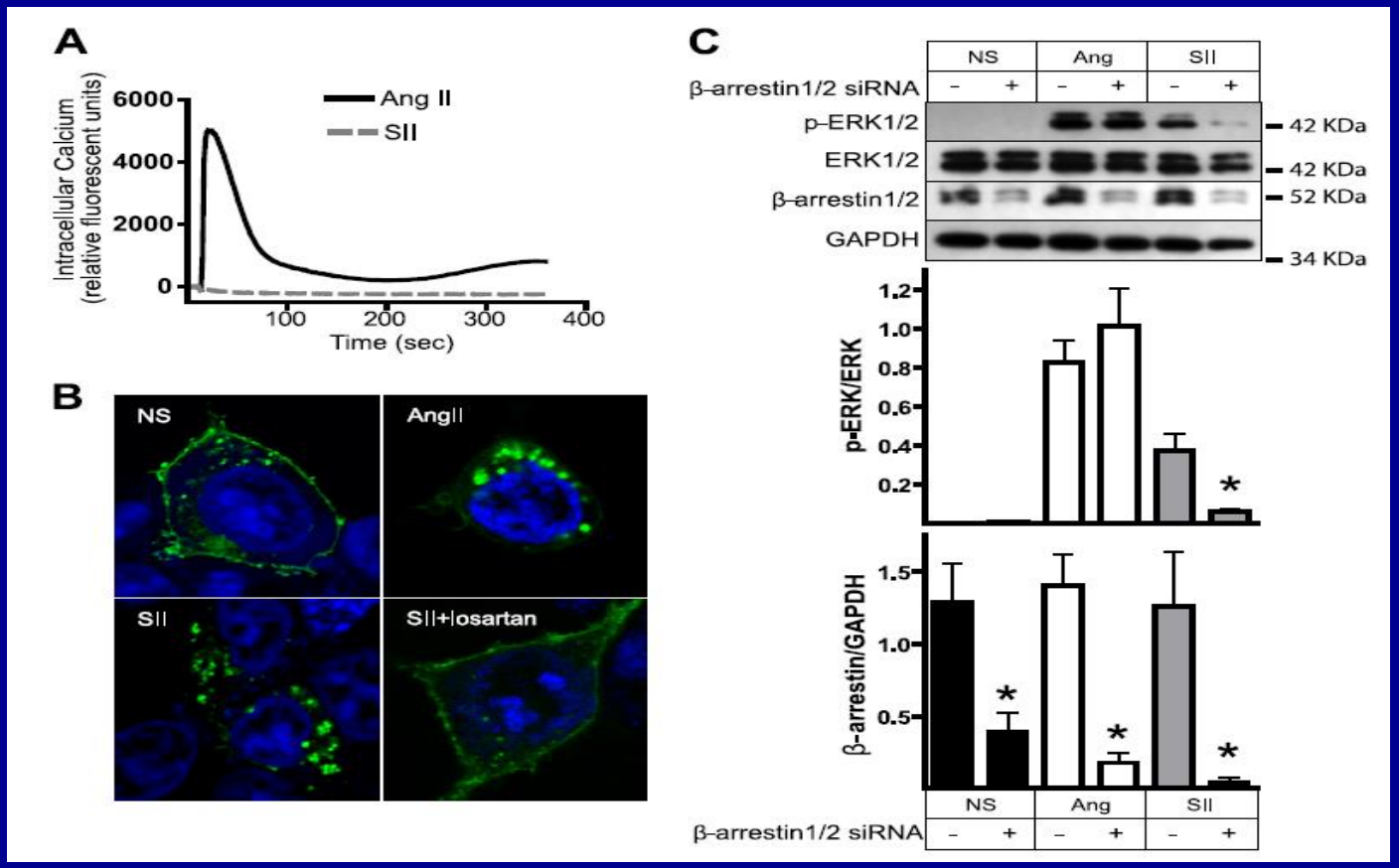
Receptor	Biased ligands	Bias
A3A adenosine	CCPA, DPMA, MRS542, MRS1760	β-Arrestin
	DBXRM	G protein
APJ (Apelin)	*Apelin 13, Apelin 36*	*Differential receptor trafficking*
AT_1	Sar1,Ile4,Ile8 Ang II (SII),	β-arrestin
	Sar1,Lys5,Ala8 Ang II (TRV120023)	
	Sar1,Tyr5,Pro7-NH2 Ang II (TRV120026)	
	Sar1,D-Ala8 Ang II (TRV120027), troglitazone	
α_{2A}-Adrenergic	*Clonidine, guanfacine*	*Differential signaling, regulation and trafficking*
	Multiple ligands	*Differential G protein signaling*
β_1-Adrenergic	Alprenolol, carvedilol	β-Arrestin
β_2-Adrenergic	Carvedilol, ICI118551, propranolol	β-Arrestin
	cyclopenylbutanephrine	
	Norepinephrin	G protein

A "biased ligand" at the AT1AR signals only through β-arrestin



Violin & Lefkowitz, TiPS 2007

β-arrestin biased agonist; SII

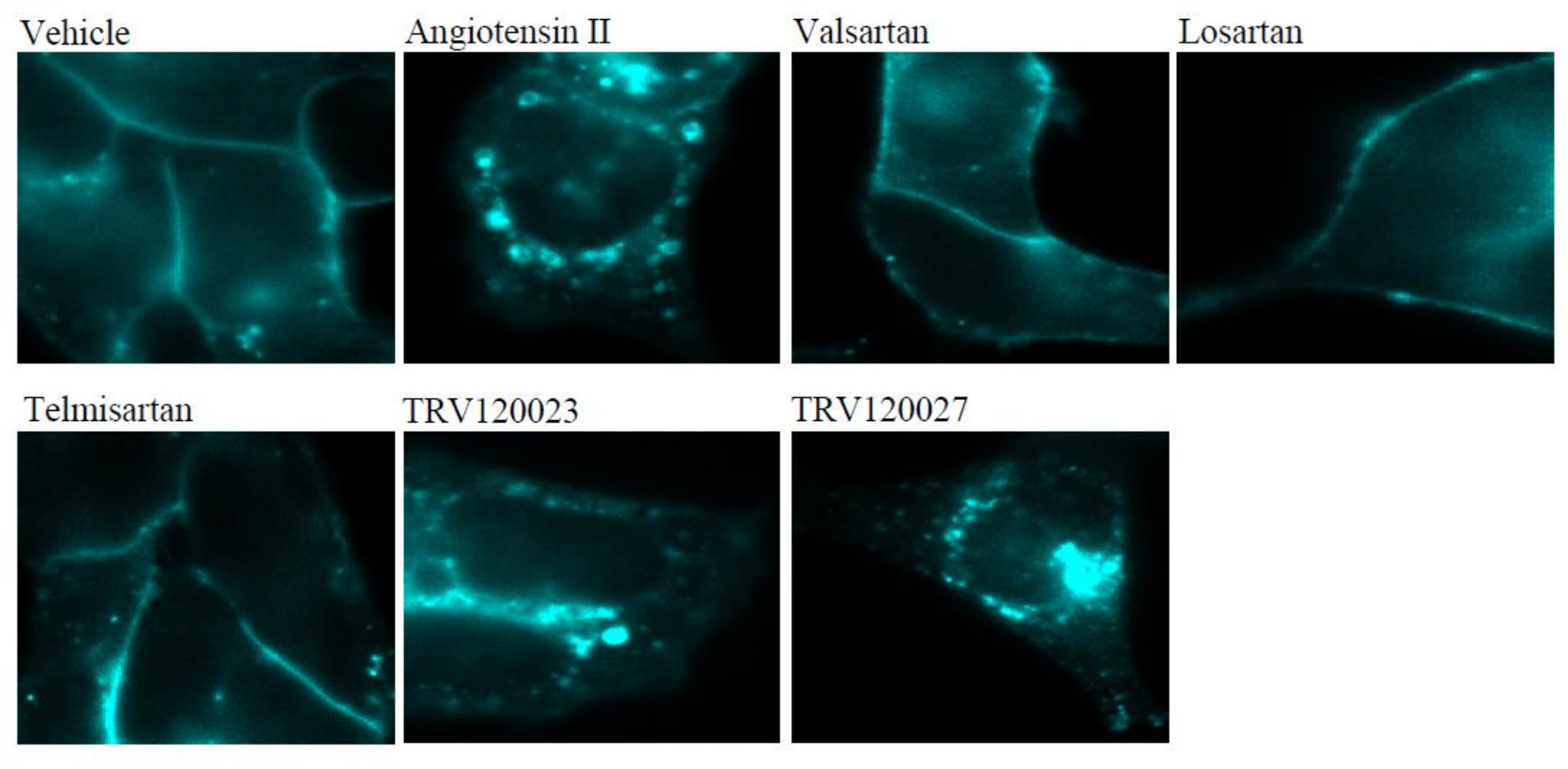


Angiotensin II induced ERK phoaphorylation ; Gq-dependent and –independent AT1R signaling

Gitte et al Molecular & Cellular Proteomics 2010

TRV Family; Evidence of Biased Agonist

TRV induced AT1R internalization

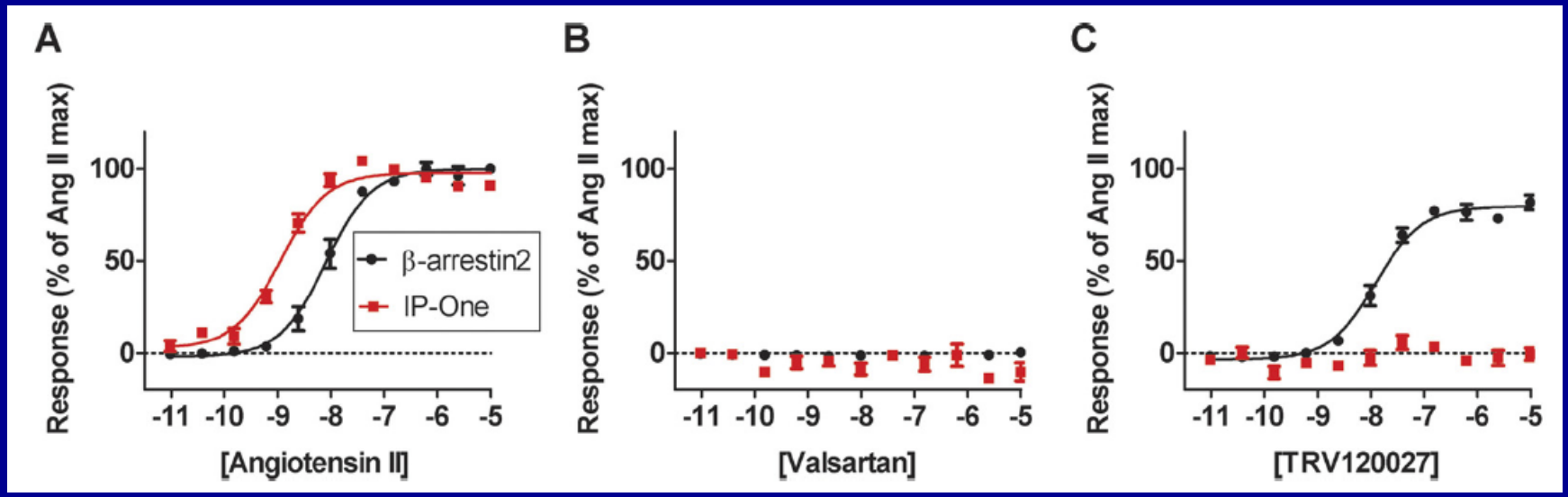


Rat AT1aR fused to cyan fluorescent protein in HEK 293 cell

Violin et al JPET 2010

TRV Family; Evidence of Biased Agonist

chemiluminescent -galactosidase activity, Red; Ang II, Black; TRV120027



TRV120023; Sar-Arg-Val-Tyr-Lys-His-Pro-Ala-OH
TRV120026; Sar-Arg-Val-Tyr-Tyr-His-Pro-NH2
TRV120027; Sar-Arg-Val-Tyr-Ile-His-Pro-D-Ala-OH

EC50 = 16nM, t1/2 = 16 min, Parenteral Use

Violin et al JPET 2010

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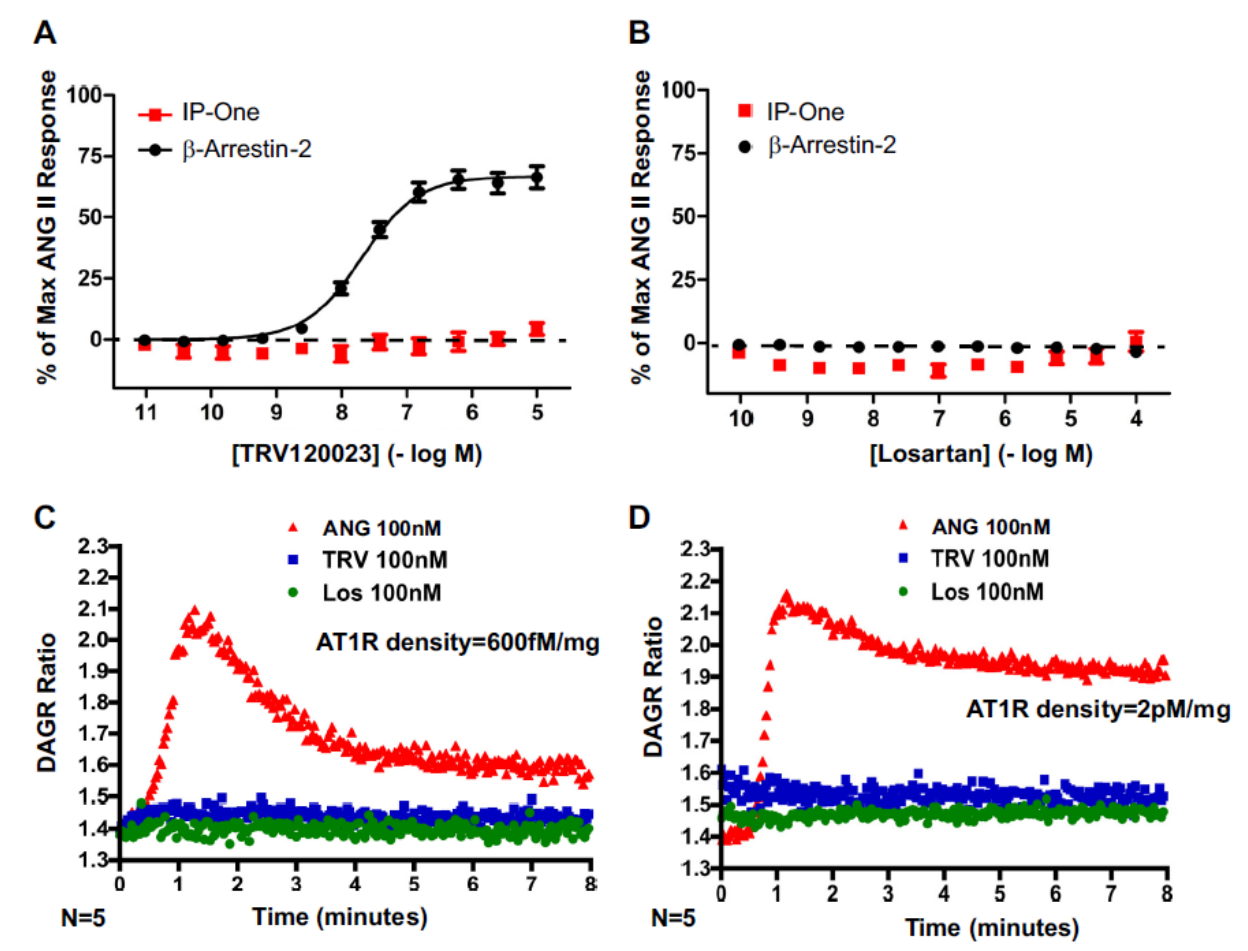

Kim KS et al, AJP-Heart Circ Physiol 2012

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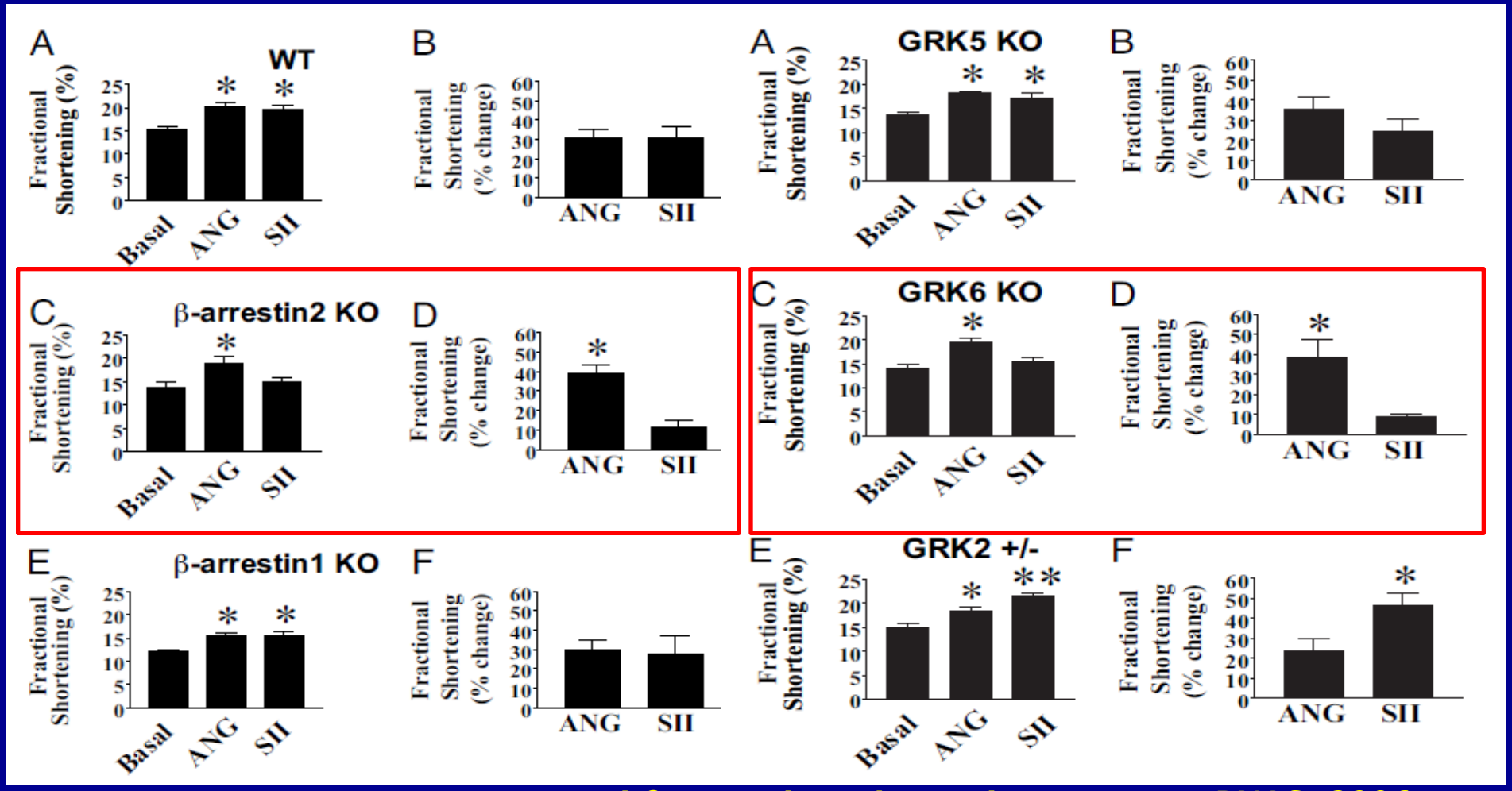
Arrestin-2 mediated inotropic effect of AT1R in isolated cardiac myocyte



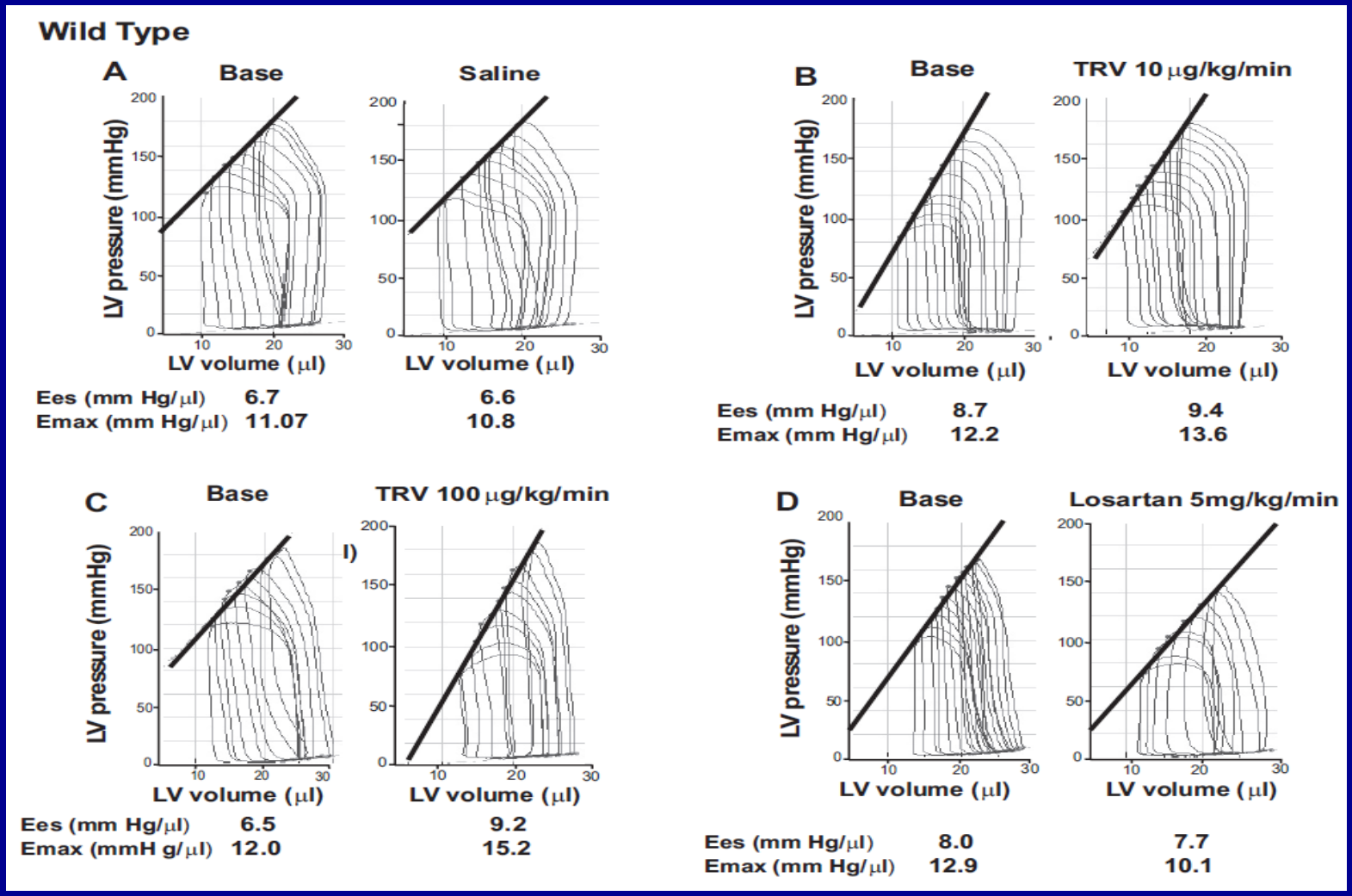
GRK6 and β-arrestin 2-dependent PNAS, 2006

Biased Agonist of AT1R

- SII
  - ^{1}Sar, ^{4}Ile, ^{8}Ile-Angiotensin II
  - Low receptor binding affinity
  - Not able to use in vivo experiment
  - No in vivo data

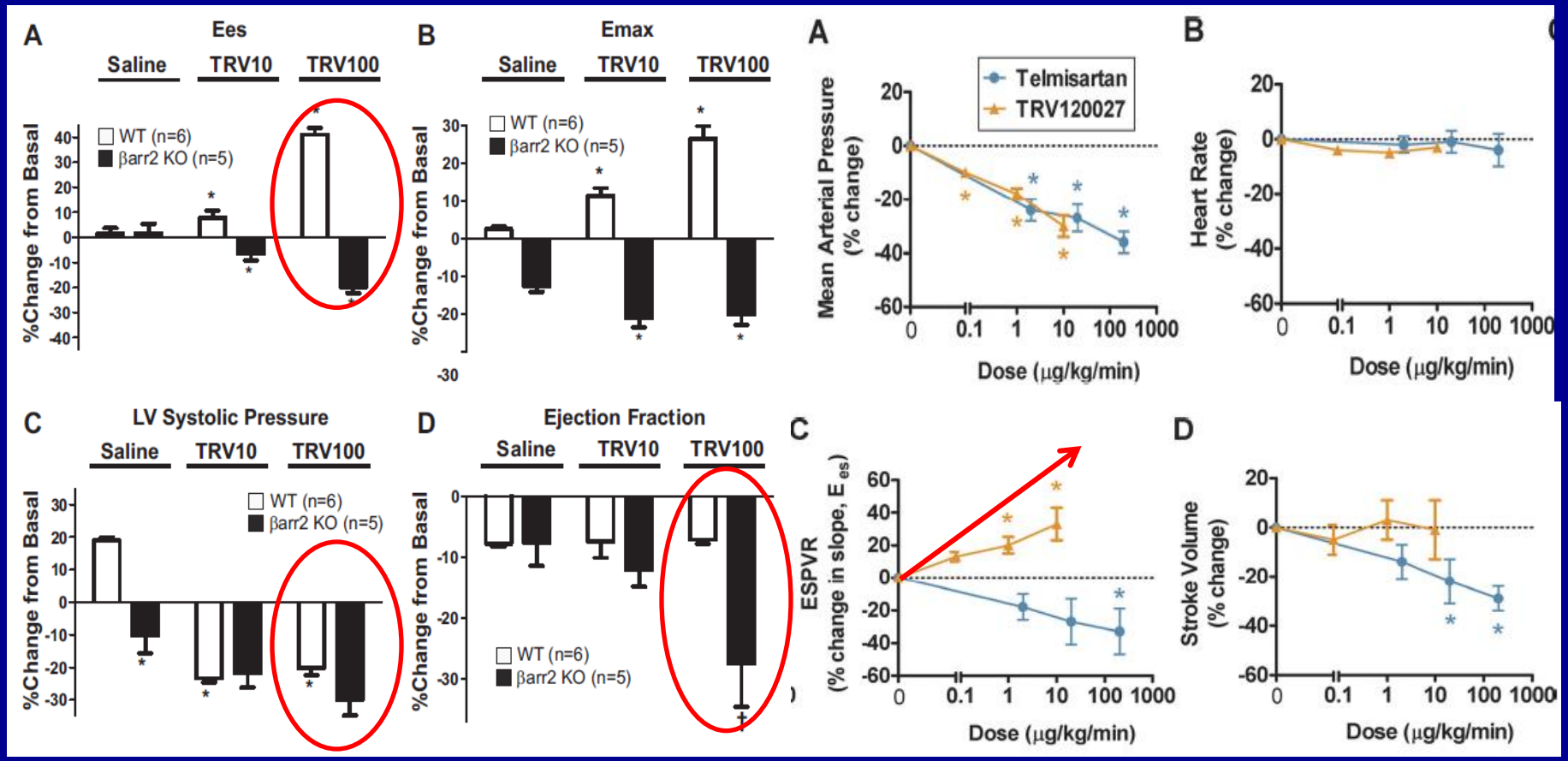
- TRV023
  - Sar-Arg-Val-Tyr-Lys-His-Pro-Ala-OH
  - MW=940.1
  - More potent β-arrestin biased agonist of AT1R

Effects on Cardiac Contractibility PV Loop Study- ESPVR (Ees)



Kim KS et al AJP-Heart Circ Physiol 2012

Enhanced contractibility and cardiac function by TRV Family



TRV120023 **TRV120027**

Violin et al JPET 2010, Kim KS et al AJP-Heart Circ Physiol 2012

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β_2-Adrenergic	Carvedilol, ICI118551, propranolol	β-Arrestin
	cyclopenylbutanephrine	
	Norepinephrin	G protein

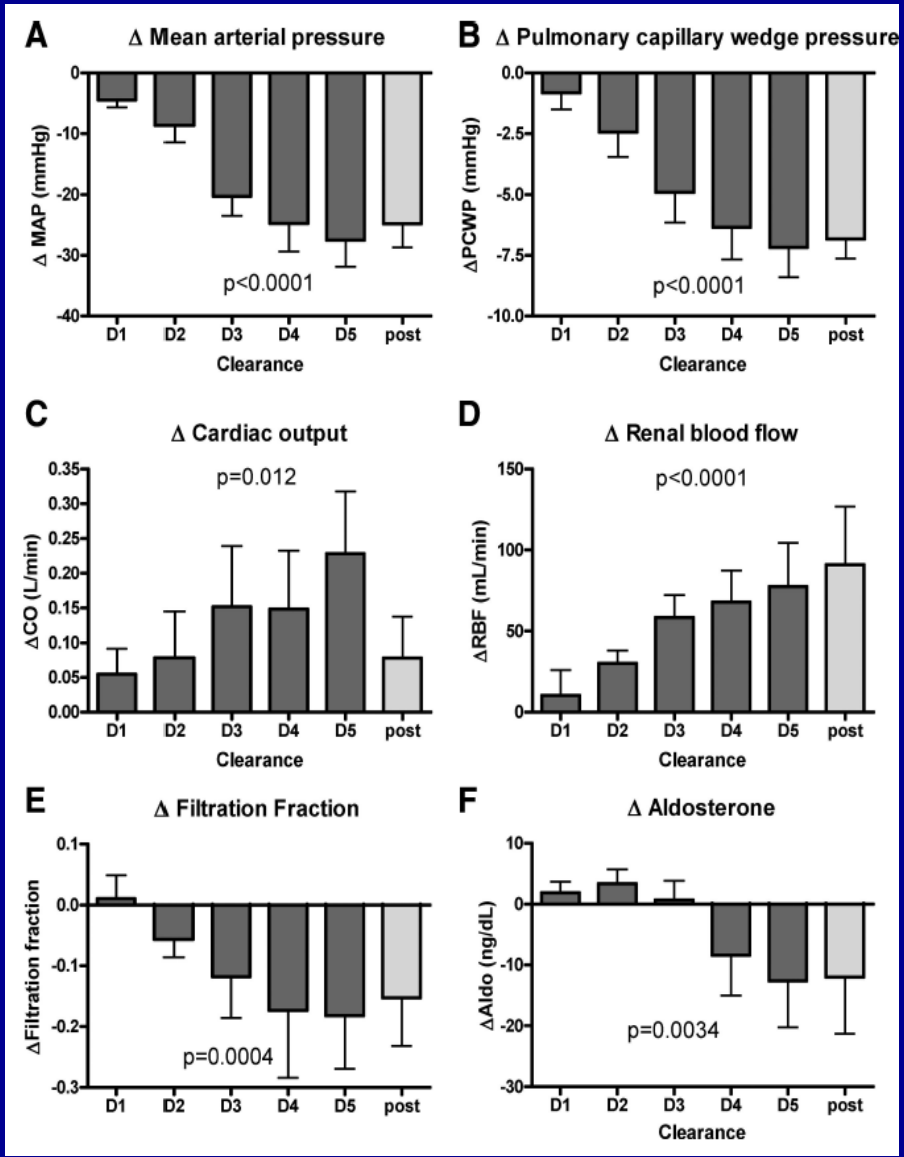
Trevena

Robert Lefkowitz M.D.

Howard Rockman M.D.

Erin Whalen Ph.D., Scott DeWire Ph.D. and Jonathan Violin, Ph.D.

TRV120027 in Canaine HF Model



MAP ↓, PCWP ↓, CO ↑
Renal blood flow ↑

No change of plasma ANP & BNP

Increased eNOS generation

Vasodilation

Reduction of cardiac preload & afterload

Boerrigter et al Circ Heart Fail 2011

TRV120027; Human Trial (First in Man)

- **To evaluate the hemodynamic effects of TRV027**
- **To evaluate the safety and tolerability of an intravenous infusion of TRV120027**

Inclusion Criteria

- Ejection fraction <35%
- NYHA class 3 or 4 HF; indication for right-heart cath
- Systolic BP >100 mmHg, heart rate ≤90 bpm at rest
- O2 sat ≥94% (RA), sCr ≤1.8 mg/dL (males) or ≤ 1.5 mg/dL (females)
- Baseline average PCWP ≥20 mmHg on 3 consecutive measures

Soergel et al, ACC 2013

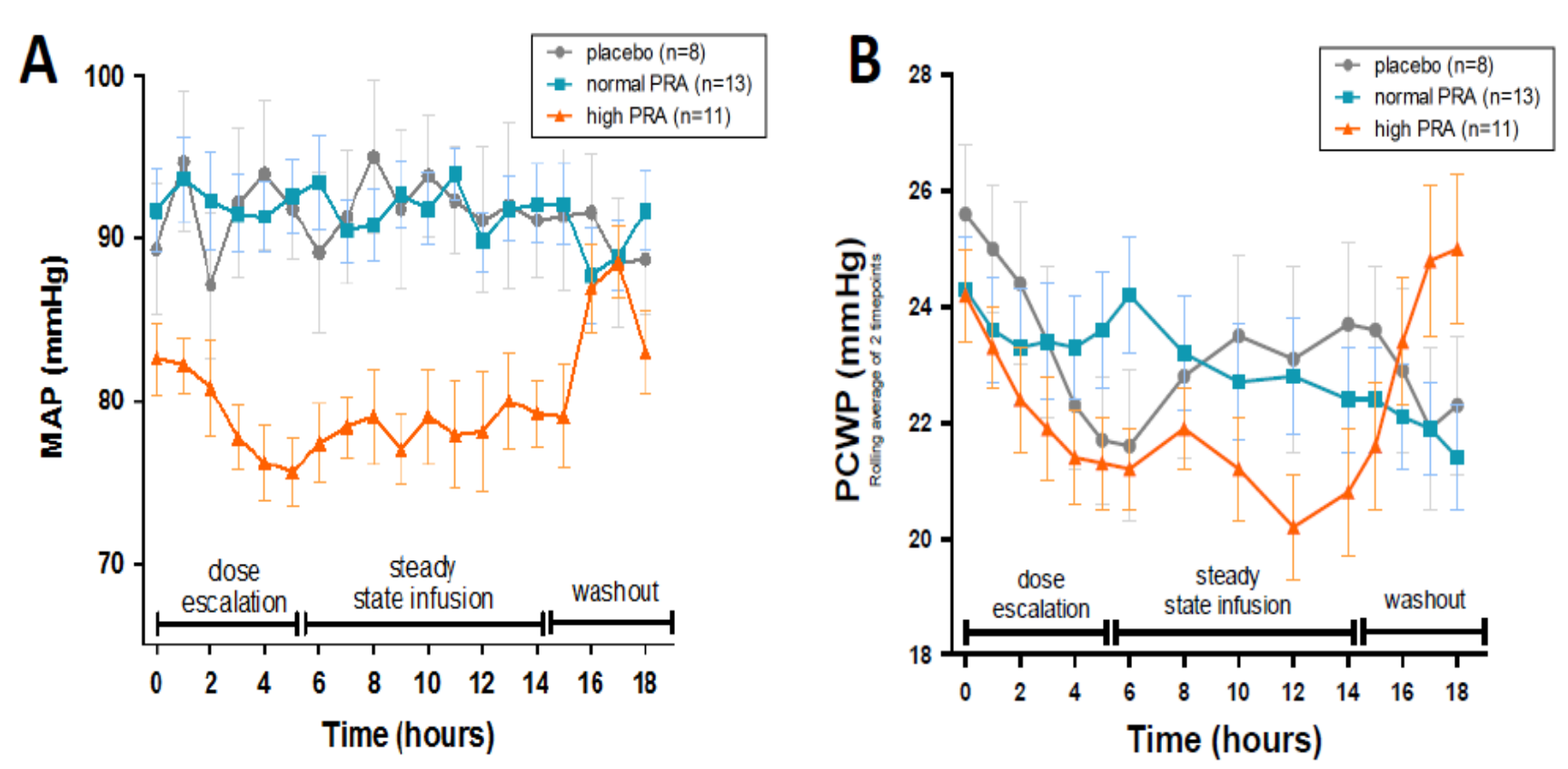
TRV120027; Human Trial (First in Man)

33 subjects participated at 15 centers in the US and Europe

Baseline characteristics	Placebo (n=8)	TRV027-treated PRA <5.82* (n=13)	TRV027-treated PRA >5.82* (n=11)
Age (years, mean, range)	59 (43-66)	55 (34-72)	56 (42-77)
EF (%, mean, range)	19 (11-35)	24 (15-35)	19 (10-30)
BNP (pg/ml, mean, range)	1511 (228-4660)	797 (46-3132)	994 (36-2806)
Baseline MAP (mmHg, mean, range)	85 (79-110)	92 (77-110)	84 (74-97)
Baseline PCWP (mmHg, mean, range)	27.4 (19.5-30.0)	24.3 (20.8-28.8)	24.2 (21.3-28.5)
Baseline PRA (ng/mL/hr, mean, range)	6.6 (0.2-25.1)	2 (0.2-5.0)	11.2 (1.5-25.2)
Baseline AngII (pg/mL, mean, range)	32.3 (16-89)	22.1 (14-44)	49 (15-149)

Soergel et al, ACC 2013

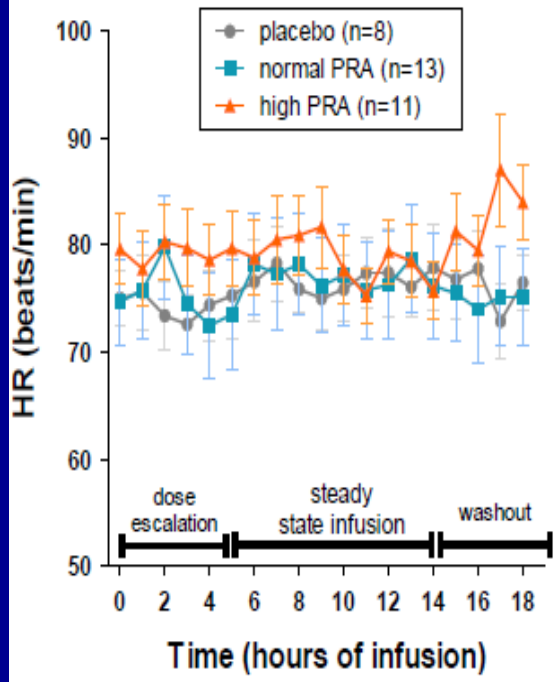
TRV027 reduces MAP and potentially PCWP in CHF subjects



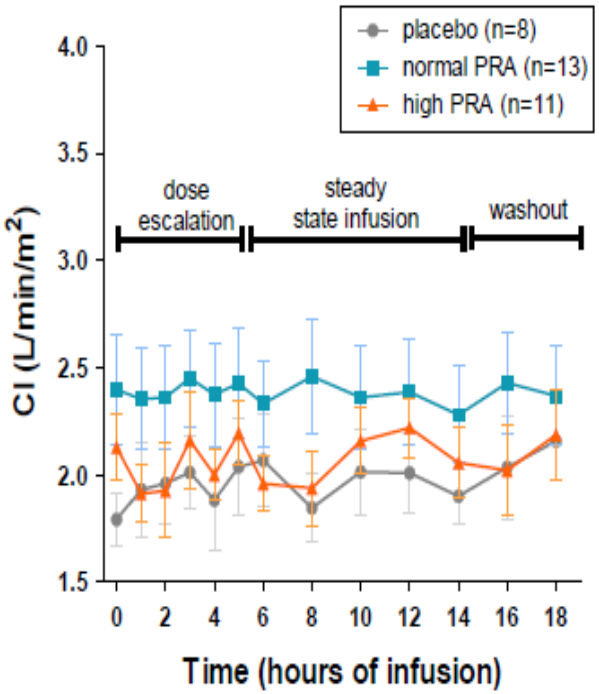
Soergel et al, ACC 2013

TRV027 did not adversely effect HR, CI, or biomarkers

A



B



C

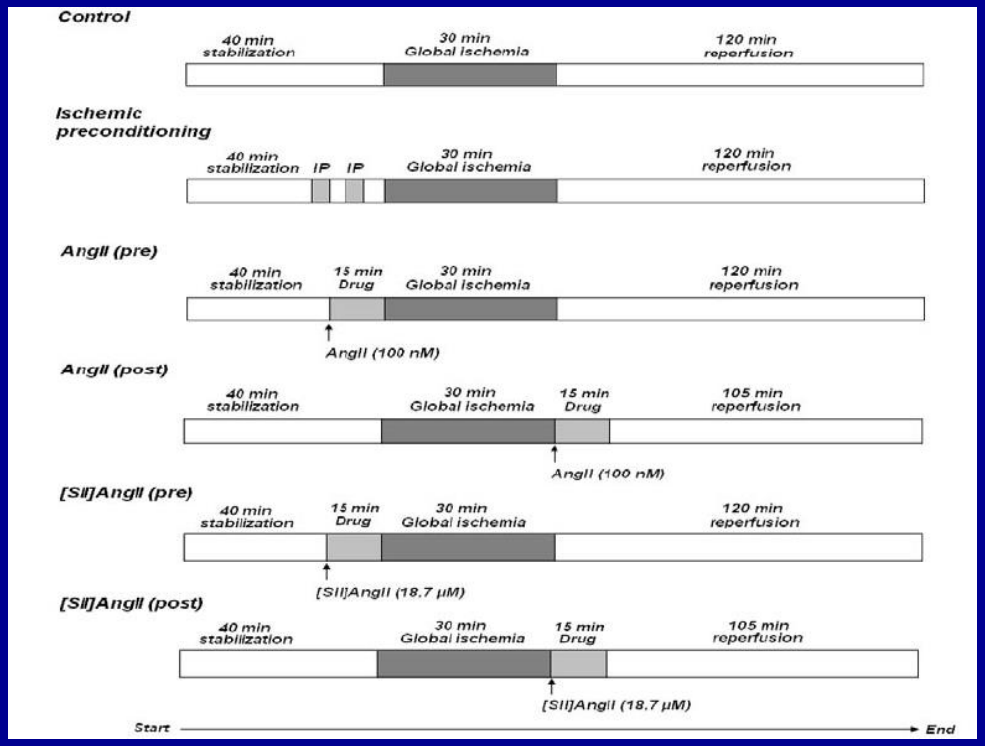
Change in biomarkers	Placebo (n=8)	TRV027-treated PRA <5.82† (n=13)	TRV027-treated PRA >5.82† (n=11)
Cystatin-c mg/L, mean (range)	0.03 (-0.1-0.2)	0.04 (-0.1-0.1)	0.01 (-0.1-0.1)
BNP pg/ml, mean (range)	276 (-1-535)	296 (-25-1061)	142 (-145-1035)
AngII pg/ml, mean (range)	-8.0 (-30--1)	0.0 (-18-17)	35.7* (-20-160)
PRA ng/mL/hr, mean (range)	-3.0 (-19.5-0.1)	-1.0 (-2.8-0.6)	7.3** (-10.4-18.4)
Creatinine mg/dL, mean (range)	-2.4 (-25-11)	1.9 (-16-16)	-3.2 (-17-10)

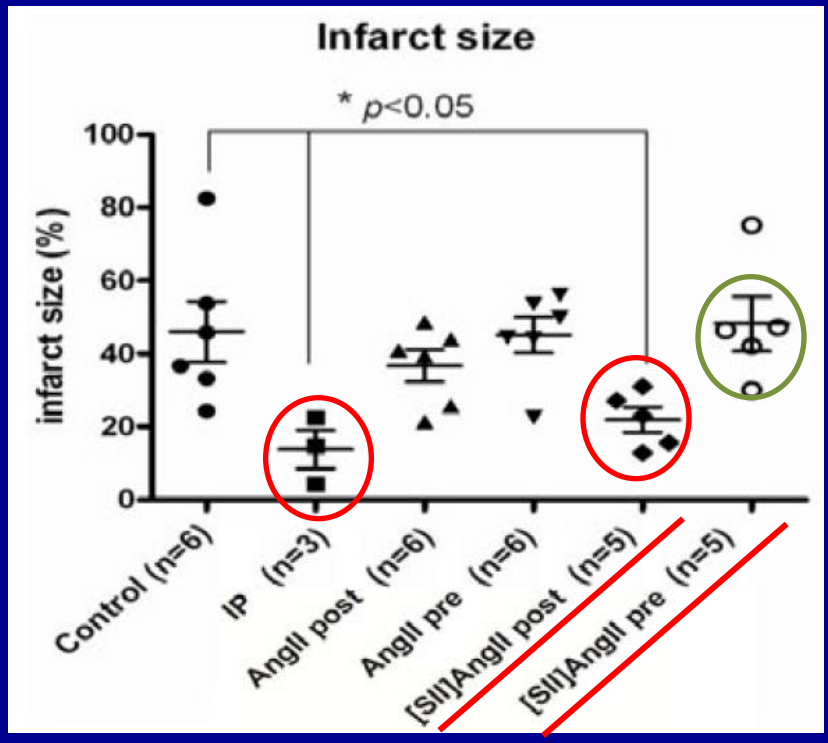
† upper limit of normal for laboratory
* high PRA significantly different than placebo (Kruskall-Wallis, $p<0.05$)
** high PRA significantly different than normal PRA (Kruskall-Wallis, $p<0.05$)

Soergel et al, ACC 2013

- GPCR in Heart
- RAS system
- Angiotensin II and AT1R
- β-arrestin
- Biased Agonism of AT1R
- AT1R β-arrestin biased Agonist
  - Effects on Cardiac Performance
  - Effects on HF model
  - Effects on Ischemia Reperfusion Injury

SII Reduced Ischemia Reperfusion Injury

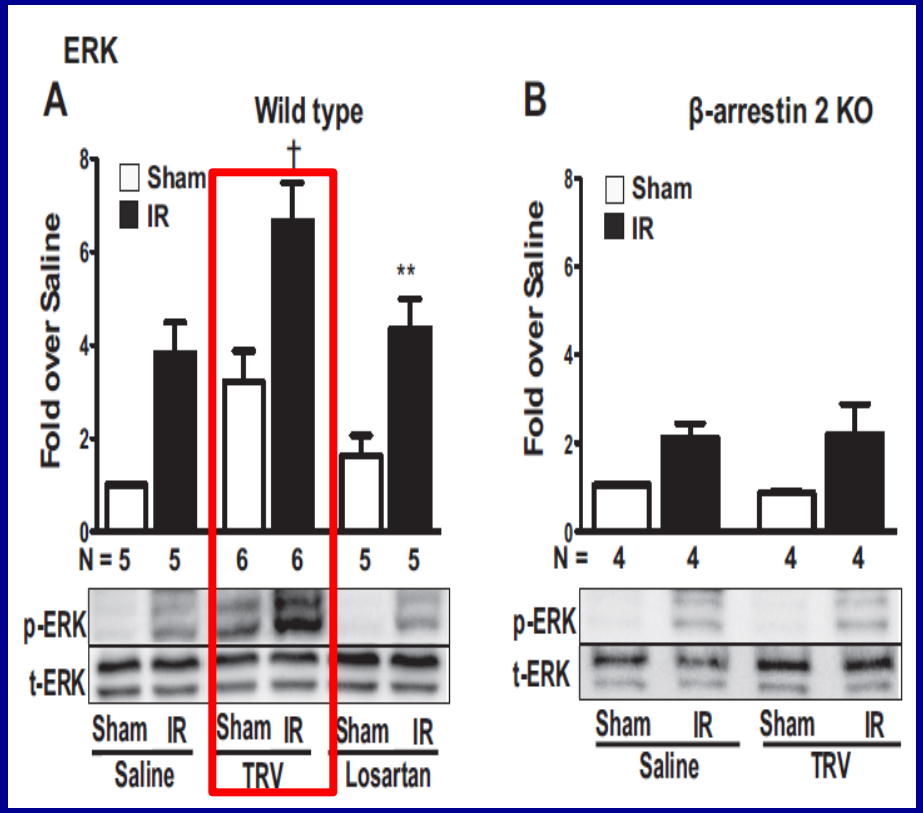


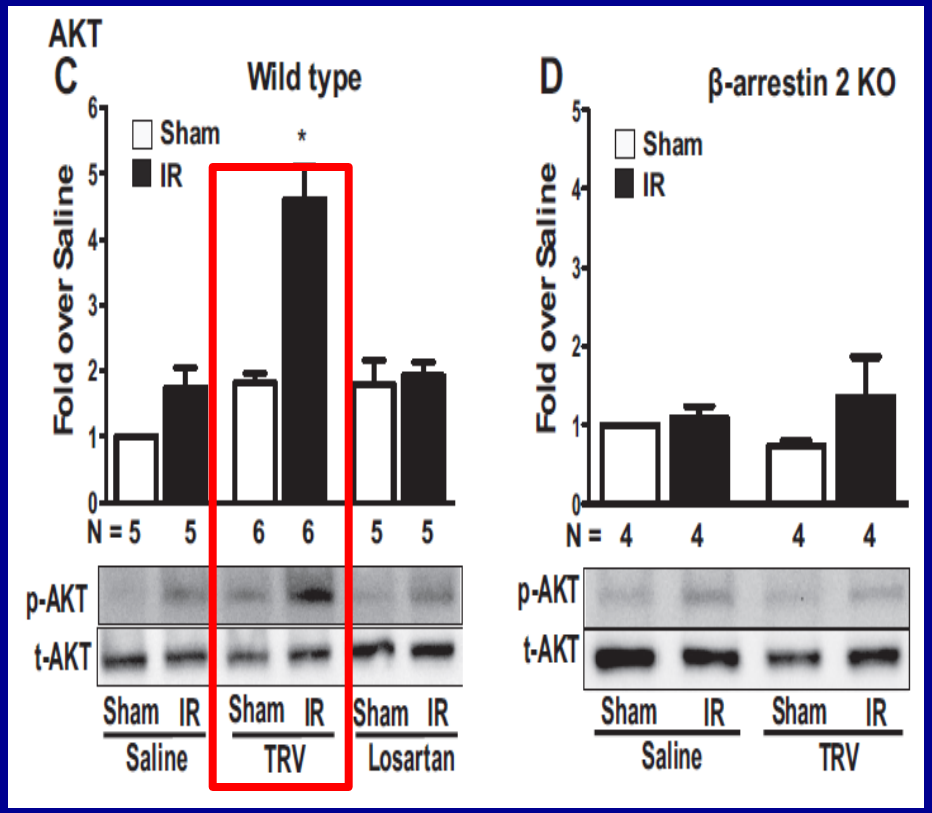


Ex vivo ischemia reperfusion injury model using langendorff system
AngII ; 100nM, [SII]AngII; 18.7 µM in Langendorff perfusion buffer

Hostrup et al Cell Physiol Biochem 2012

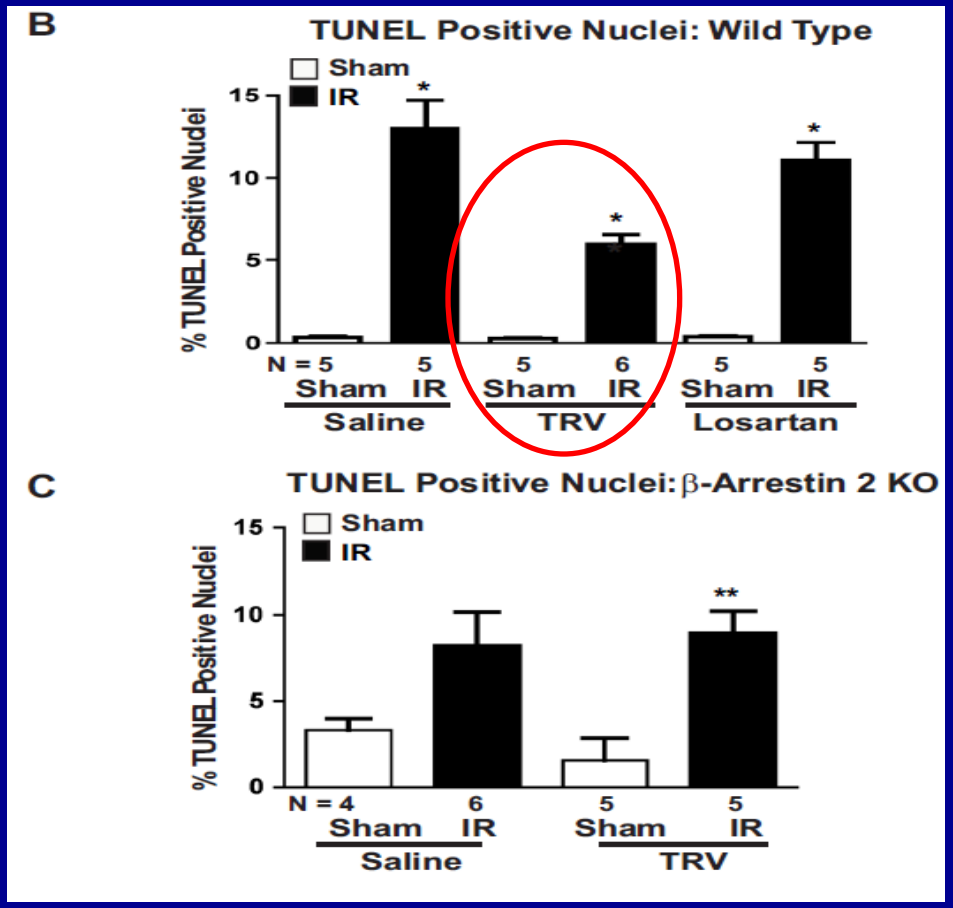
TRV120023 enhances ERK1/2 and Akt signaling after ischemia reperfusion (IR)

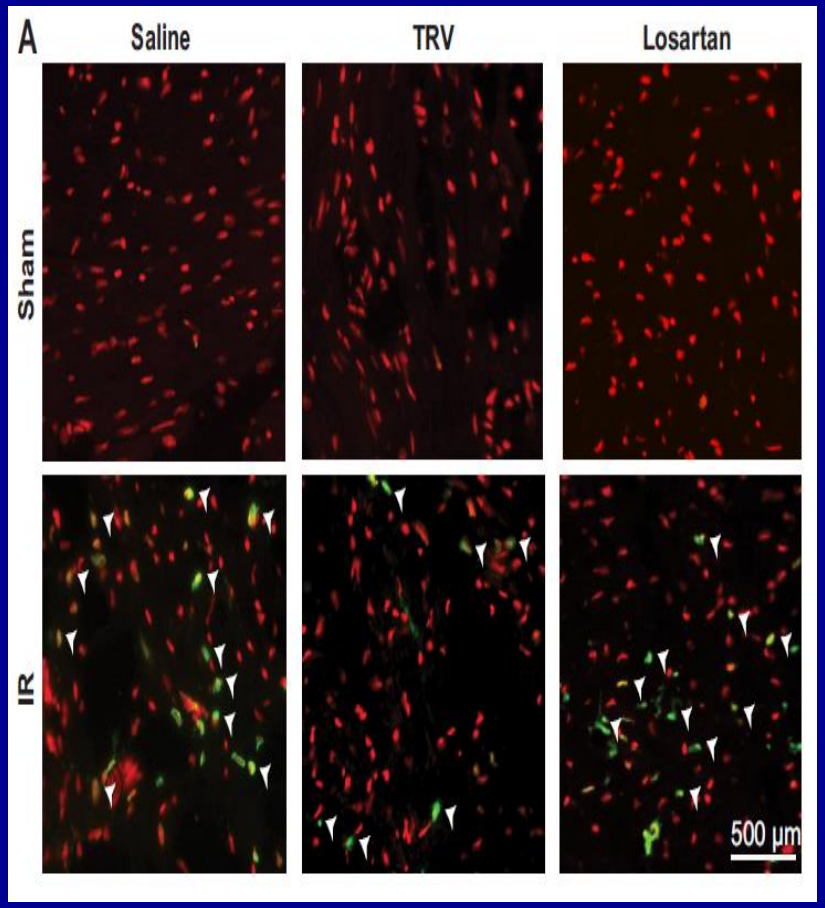




Kim KS et al AJP-Heart Circ Physiol 2012

IR-induced cardiomyocyte apoptosis was attenuated by TRV120023 pretreatment





Kim KS et al AJP-Heart Circ Physiol 2012

Summary

- AT1R β-arrestin biased Agonist
- Increase in cardiac performance and preserved stroke volume
- Decreased preload and afterload in HF
- Decreased apoptosis during acute cardiac injury

Conclusion

- β-arrestin biased agonism of AT1R
  - Enhanced cardiac performance
  - Cardioprotective effect during acute cardiac injury

- Target of new drug development in cardiovascular disease

Thank You for Your Attention