Molecular mechanisms controlling vascular smooth muscle contractility

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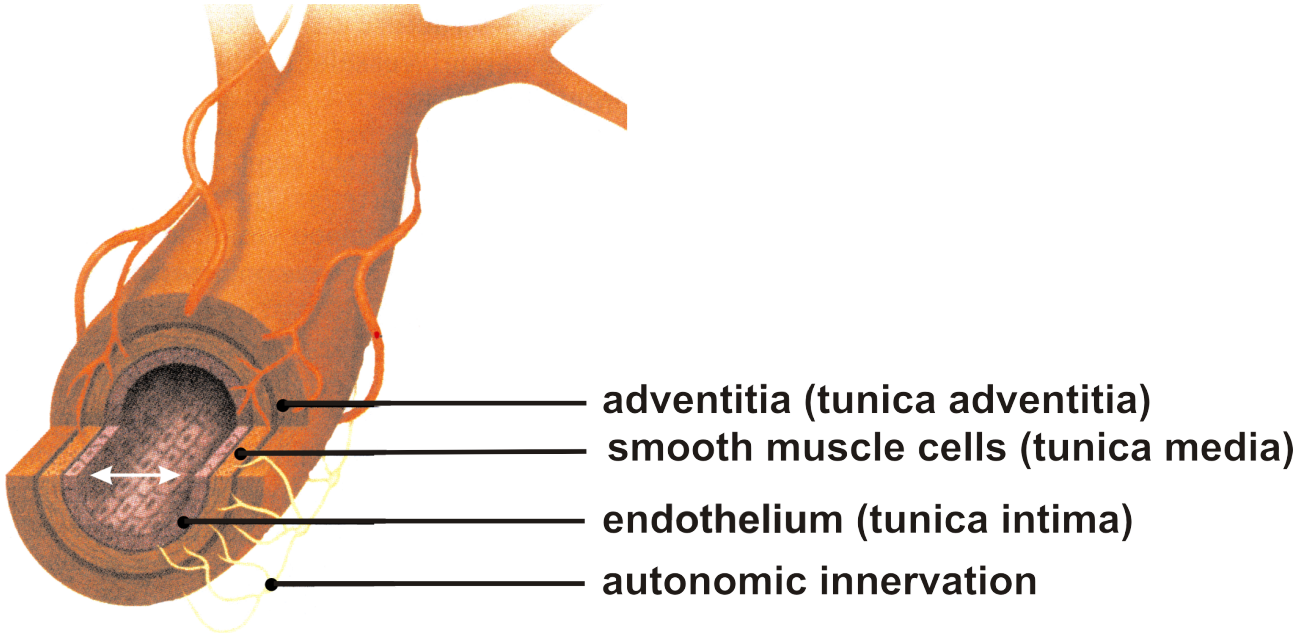
Mechanisms controlling diameter of arterial resistance vessels



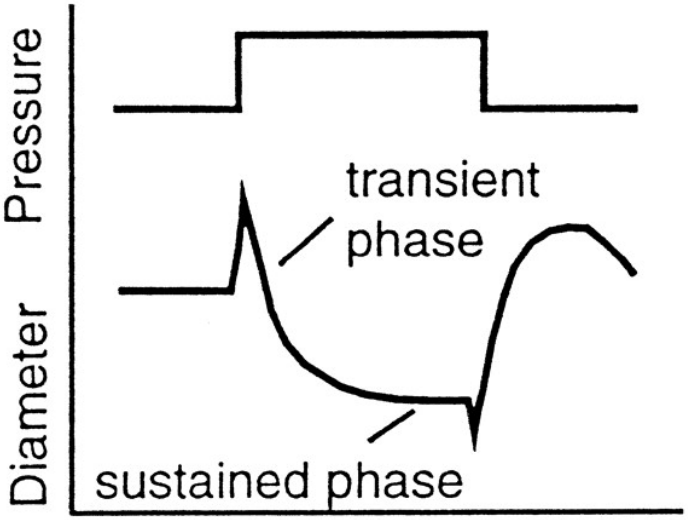
INTRINSIC MECHANISMS

Membrane potential
Ca^{2+}-sensitization

EXTRINSIC MECHANISMS

Neurotransmitters
Circulating hormones
Endothelial factors
Electrical coupling via
myoendothelial gap junctions
Local factors from parenchyma

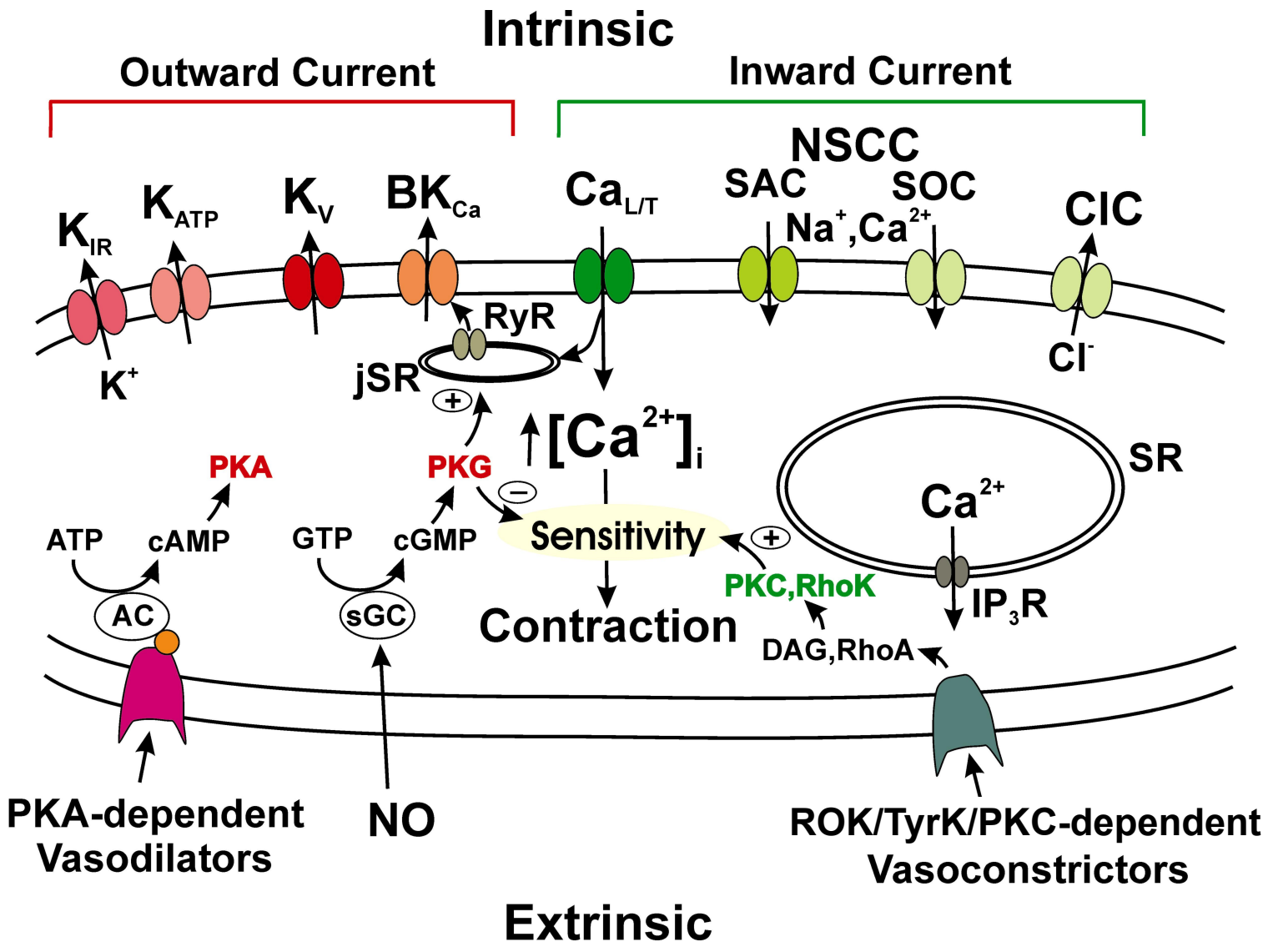
Myogenic tone in vascular smooth muscle resistance arteries



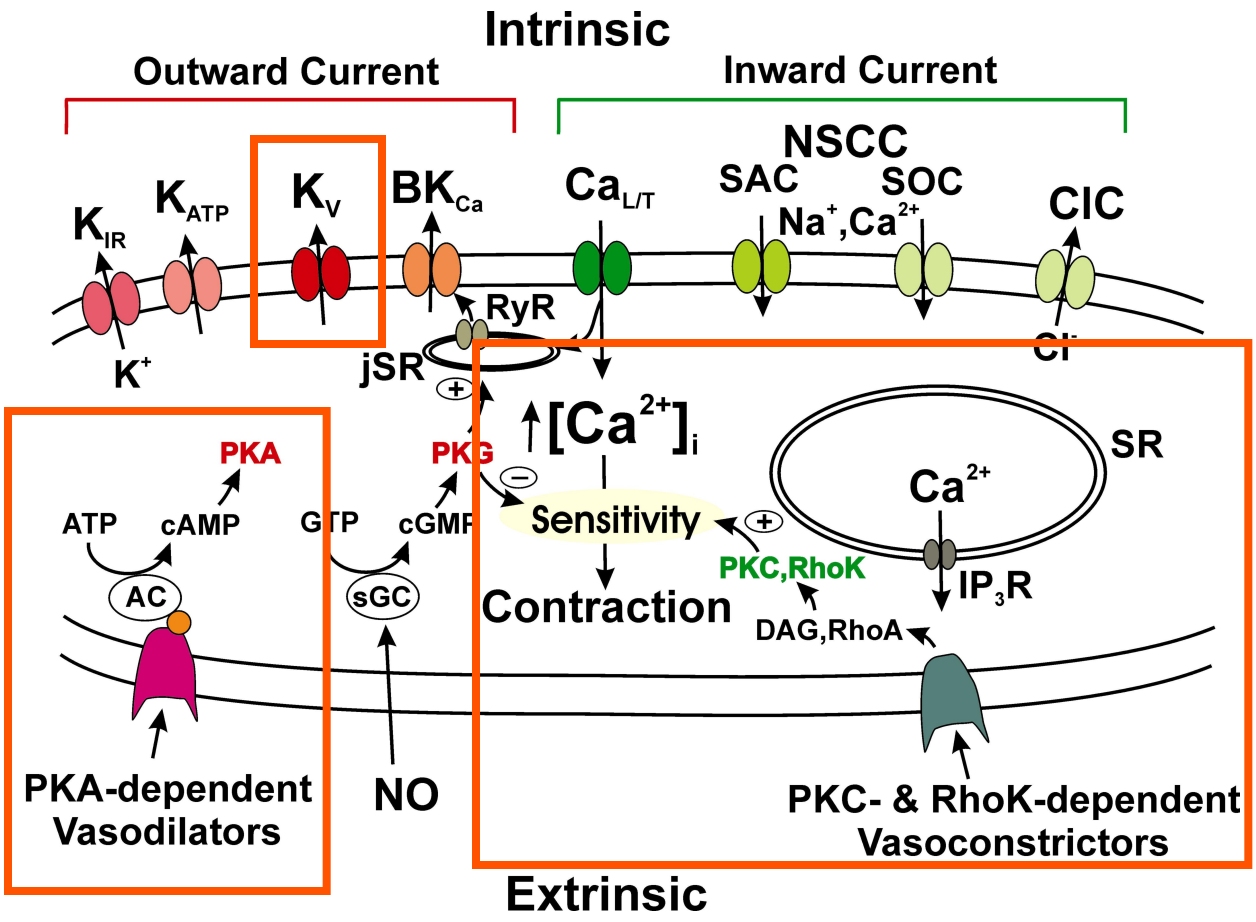
- **Mechanisms are intrinsic to vascular smooth muscle (does not require neurohormonal or endothelial input)**

- **Important determinant of:**
  1. **Peripheral vascular resistance and blood pressure**
  2. **Capillary pressure**
  3. **Local autoregulation of blood flow**

Regulation of vascular smooth muscle contraction



Objectives



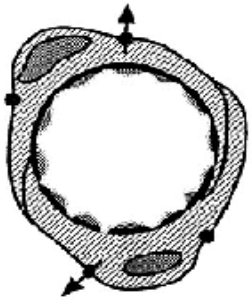
1. Molecular composition of smooth muscle Kv channels in the mesenteric vasculature

2. Molecular basis for regulation of Kv1.2 by PKA

3. Role of ROK-dependent Ca^{2+} sensitization in myogenic control of arterial diameter in the cerebral vasculature

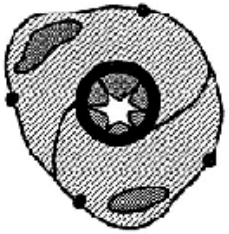
Consequences of dysfunctional control of VSM tone

Abnormal depolarization
↓ K^+ channel activity
↑ myogenic responsiveness
↑ ROK activity
↑ Ca^{2+} sensitization

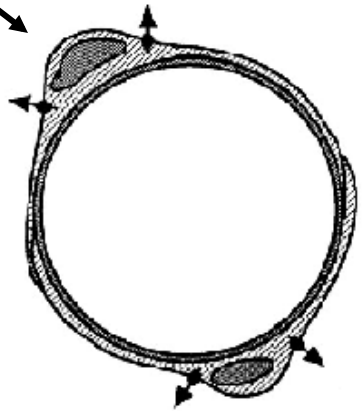


arteriole

Abnormal hyperpolarization
↑ K^+ channel activity
↓ myogenic responsiveness
↓ ROK activity
↓ Ca^{2+} sensitization



- **hypertension**
- **vasospasm**
- **type II diabetes**

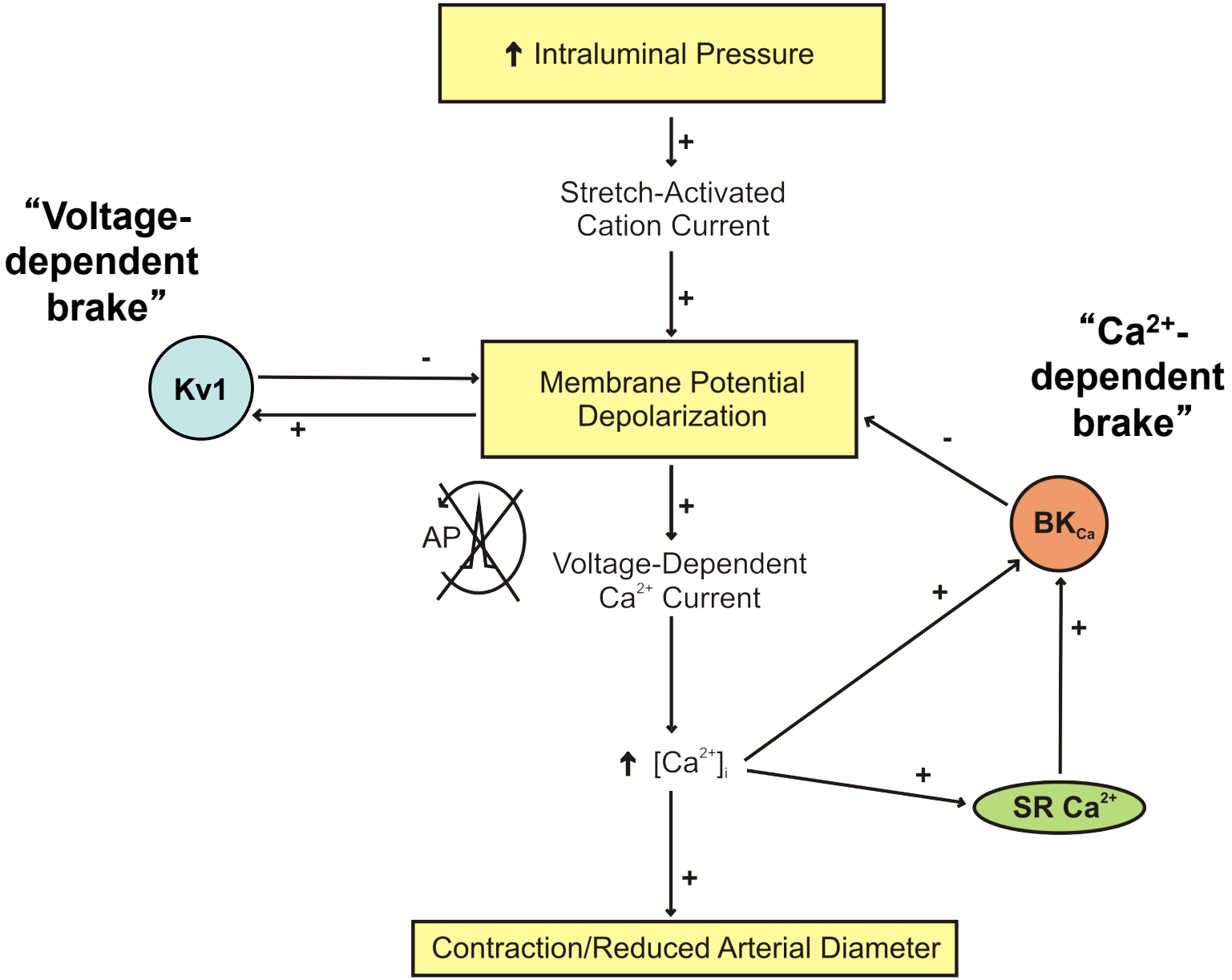


- **endotoxic shock**
- **hemorrhagic shock**
- **diabetic end-stage renal failure**

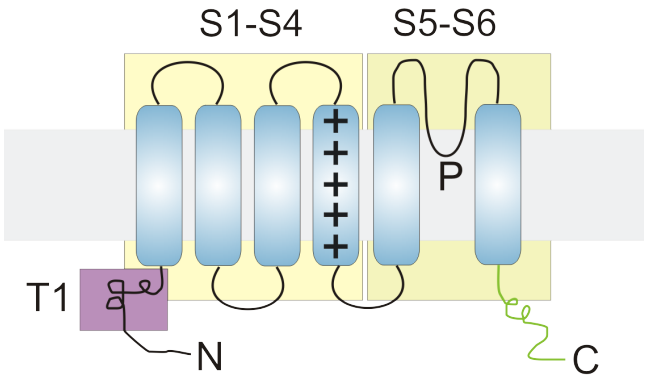
Part 1:

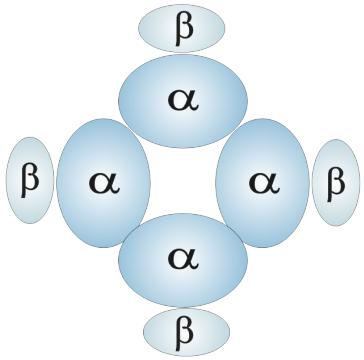
Molecular composition of vascular smooth muscle Kv1 channels in the mesenteric vasculature

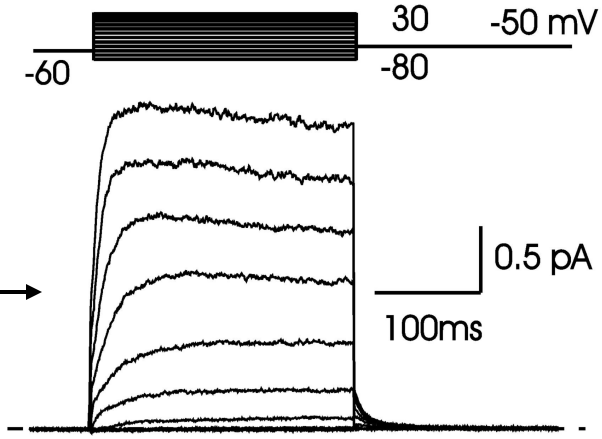
Negative-feedback regulation of myogenic depolarization



Kv channel structure







VSM K_{DR} current

=sum of current flowing through1+ Kv channels of varying composition

Kv superfamily of pore-forming α subunits

Kv1	1.1-1.8
Kv2	2.1,2.2
Kv3	3.1-3.4
Kv4	4.1-4.3
Kv5*	5.1
Kv6*	6.1-6.4
Kv7*	7.1-7.5
Kv8*	8.1,8.2
Kv9*	9.1-9.3
Kv10	10.1,10.2
Kv11	
Kv12	

Kv superfamily of modulatory β subunits

Kv 1 β	1.1-1.3
Kv 2 β	2.1,2.2
Kv 3 β	3.1

Kv transcript and protein expression in mesenteric arteries

Kv subunit	Transcript Expression	Protein Expression
Kv1.1	+/- (3/6)	-
Kv1.2	+	+
Kv1.4	+	+
Kv1.5	+	+
Kv1.6	+	+
Kvβ1.1	+	x
Kvβ1.2	+	x
Kvβ1.3	+	x
Kvβ2.1	+	x

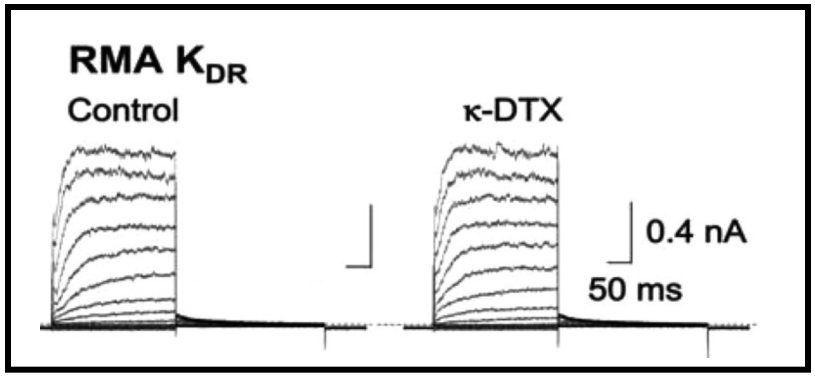
+ detected

- not detected

x not determined

Kv transcript and protein expression in mesenteric arteries

Kv subunit	Transcript Expression	Protein Expression
~~Kv1.1~~	~~+/- (3/6)~~	~~-~~
Kv1.2	+	+
Kv1.4	+	+
Kv1.5	+	+
Kv1.6	+	+
Kvβ1.1	+	x
Kvβ1.2	+	x
Kvβ1.3	+	x
Kvβ2.1	+	x



***κ-dendrotoxin – blocks channels containing 1+ Kv1.1 α-subunits**

\+ detected

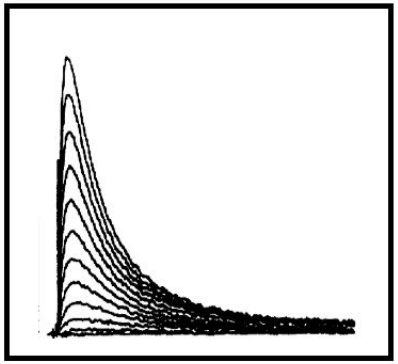
\- not detected

x not determined

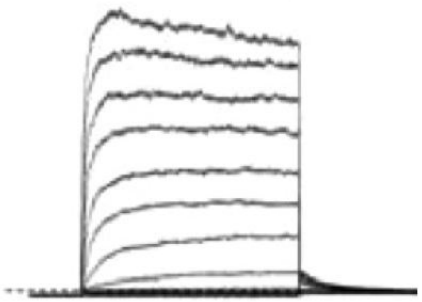
Kv transcript and protein expression in mesenteric arteries

Kv subunit	Transcript Expression	Protein Expression
~~Kv1.1~~	~~+/- (3/6)~~	~~-~~
Kv1.2	+	+
~~Kv1.4~~	~~+~~	~~+~~
Kv1.5	+	+
Kv1.6	+	+
Kvβ1.1	+	x
Kvβ1.2	+	x
Kvβ1.3	+	x
Kvβ2.1	+	x

Kv1.4
Kv1.4/Kv1.2
Kv1.4/Kv1.5



RMA K_{DR}



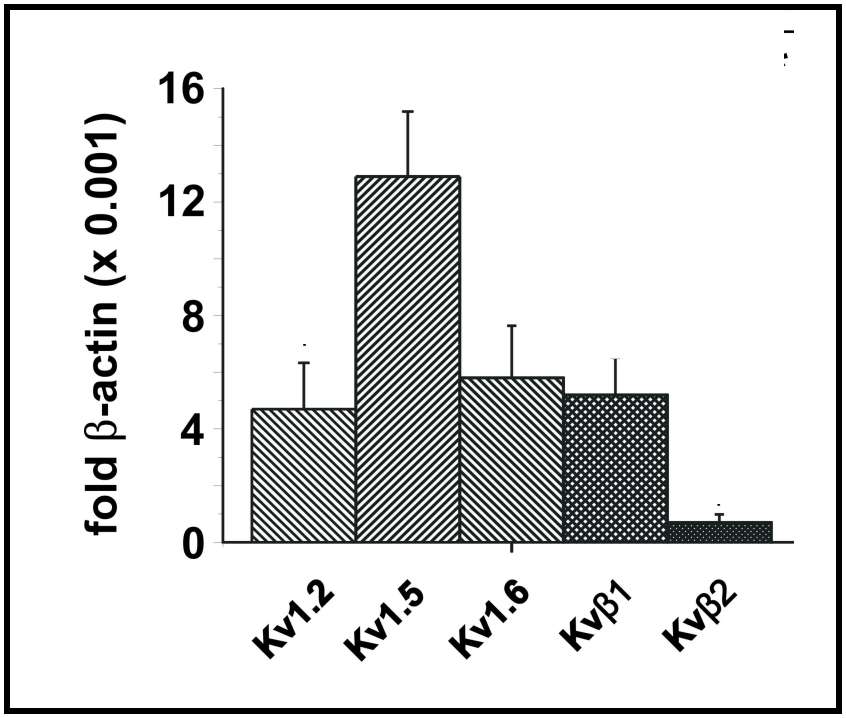
+ detected

- not detected

x not determined

Kv transcript and protein expression in mesenteric arteries

Kv subunit	Transcript Expression	Protein Expression
~~Kv1.1~~	~~+/- (3/6)~~	~~-~~
Kv1.2	+	+
~~Kv1.4~~	~~+~~	~~+~~
Kv1.5	+	+
Kv1.6	+	+
Kvβ1.1	+	x
Kvβ1.2	+	x
Kvβ1.3	+	x
~~Kvβ2.1~~	~~+~~	~~x~~



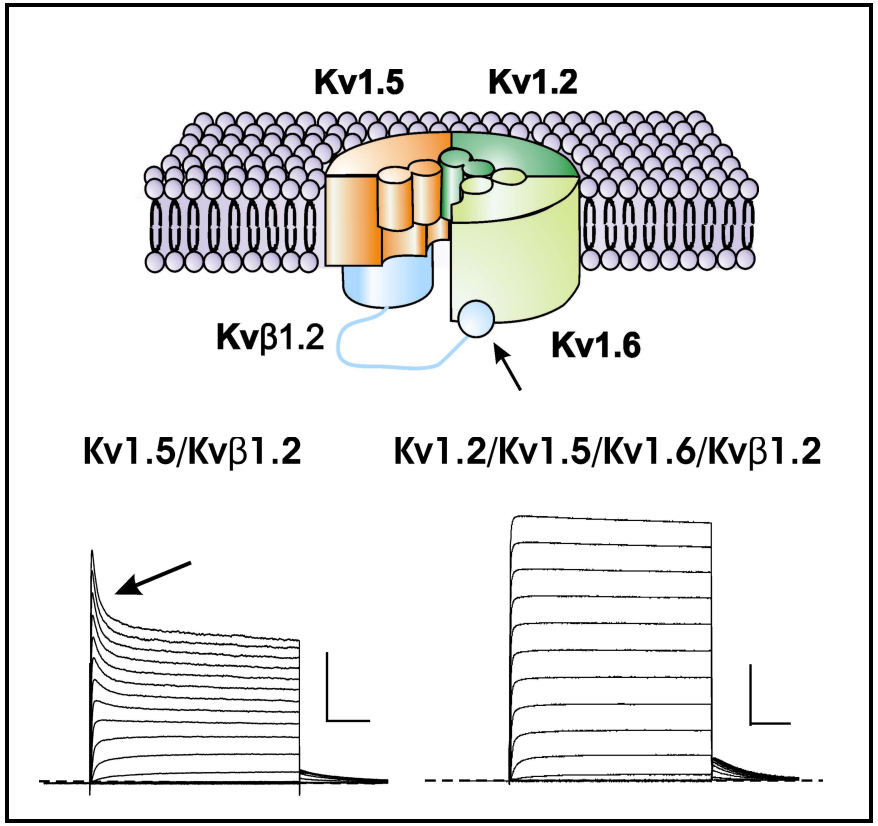
+ detected

- not detected

x not determined

Kv transcript and protein expression in mesenteric arteries

Kv subunit	Transcript Expression	Protein Expression
~~Kv1.1~~	~~+/- (3/6)~~	~~-~~
Kv1.2	+	+
~~Kv1.4~~	~~+~~	~~+~~
Kv1.5	+	+
Kv1.6	+	+
Kvβ1.1	+	x
Kvβ1.2	+	x
Kvβ1.3	+	x
~~Kvβ2.1~~	~~+~~	~~x~~



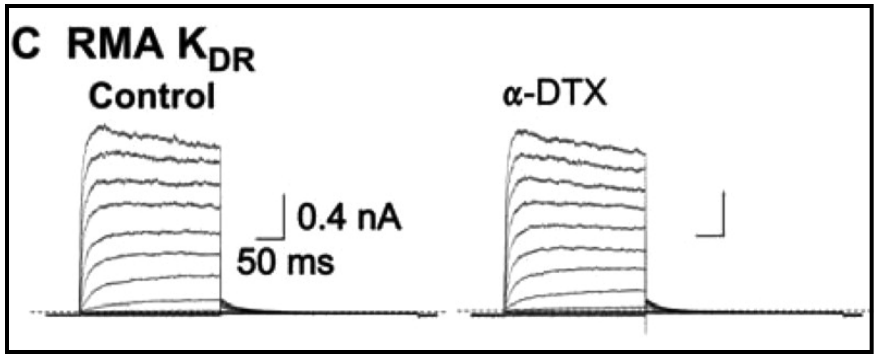
+ detected

- not detected

x not determined

Kv transcript and protein expression in mesenteric arteries

Kv subunit	Transcript Expression	Protein Expression
~~Kv1.1~~	~~+/- (3/6)~~	~~-~~
Kv1.2	+	+
~~Kv1.4~~	~~+~~	~~+~~
Kv1.5	+	+
Kv1.6	+	+
Kvβ1.1	+	x
Kvβ1.2	+	x
Kvβ1.3	+	x
~~Kvβ2.1~~	~~+~~	~~x~~



***α-dendrotoxin blocks any Kv1 channels EXCEPT those containing Kv1.5**

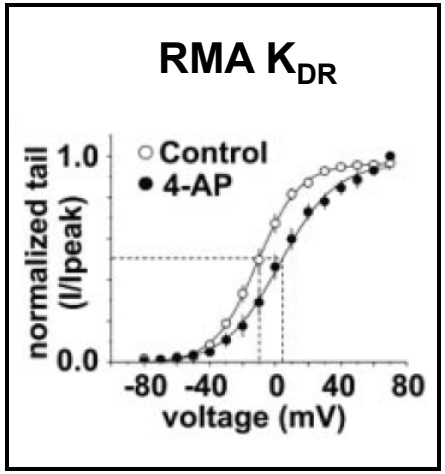
\+ detected

\- not detected

x not determined

Kv transcript and protein expression in mesenteric arteries

Kv subunit	Transcript Expression	Protein Expression
~~Kv1.1~~	~~+/- (3/6)~~	~~-~~
Kv1.2	+	+
~~Kv1.4~~	~~+~~	~~+~~
Kv1.5	+	+
Kv1.6	+	+
Kvβ1.1	+	x
Kvβ1.2	+	x
Kvβ1.3	+	x
~~Kvβ2.1~~	~~+~~	~~x~~



Kv1.5 alone – no shift ✗
Kv1.2 + Kv1.5 – shift ✓

+ detected

- not detected

x not determined

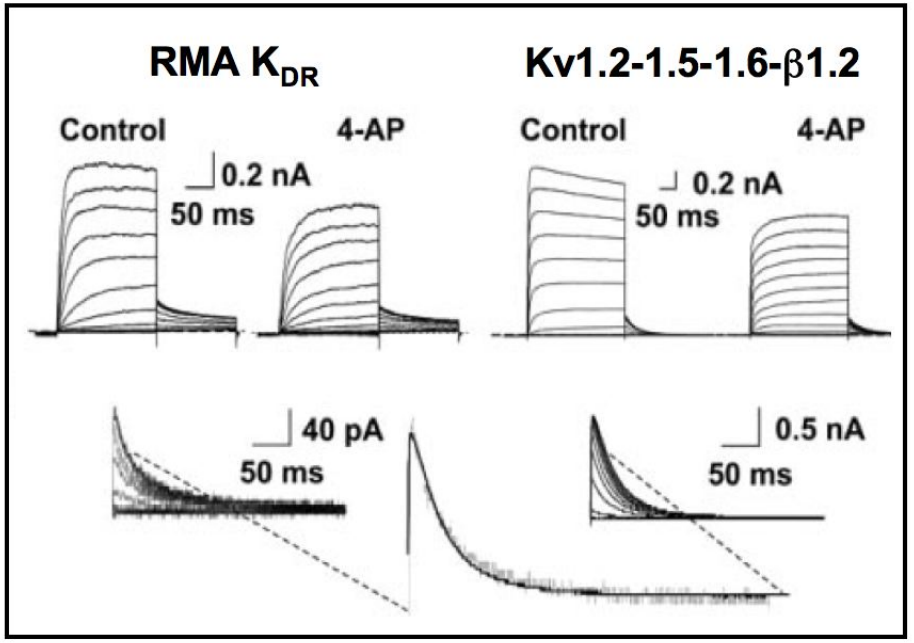
Kv transcript and protein expression in mesenteric arteries

Kv subunit	Transcript Expression	Protein Expression
~~Kv1.1~~	~~+/- (3/6)~~	~~-~~
Kv1.2	+	+
~~Kv1.4~~	~~+~~	~~+~~
Kv1.5	+	+
Kv1.6	+	+
Kvβ1.1	+	x
Kvβ1.2	+	x
Kvβ1.3	+	x
~~Kvβ2.1~~	~~+~~	~~x~~

+ detected

- not detected

x not determined



4-AP sensitivity ✓
Activation time constants (C➔O) ✓
Deactivation time constants (O➔C) ✓
Inactivation time constants (O➔I) ✓

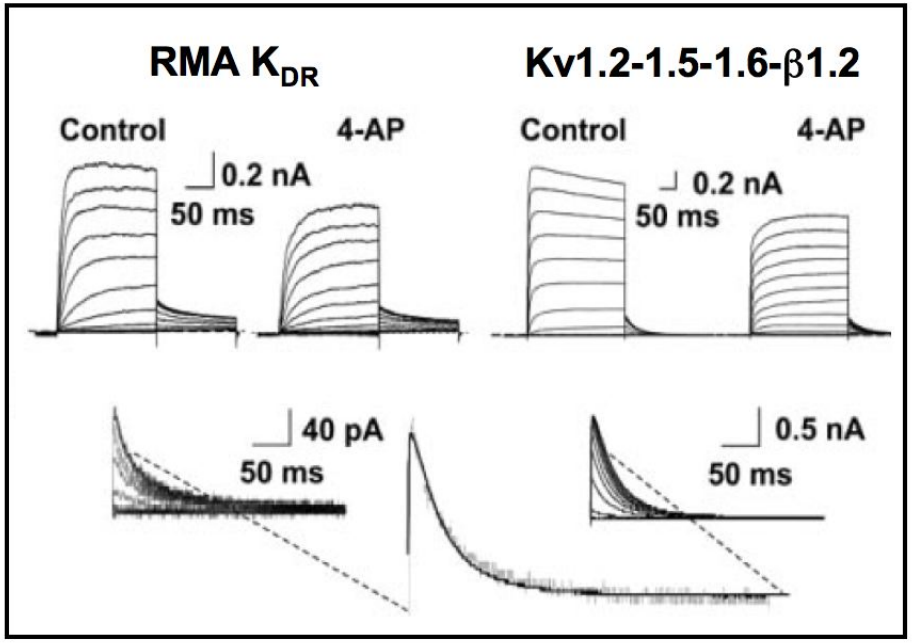
Kv transcript and protein expression in mesenteric arteries

Kv subunit	Transcript Expression	Protein Expression
~~Kv1.1~~	~~+/- (3/6)~~	~~-~~
Kv1.2	+	+
~~Kv1.4~~	~~+~~	~~+~~
Kv1.5	+	+
Kv1.6	+	+
Kvβ1.1	+	x
Kvβ1.2	+	x
Kvβ1.3	+	x
~~Kvβ2.1~~	~~+~~	~~x~~

+ detected

- not detected

x not determined



4-AP sensitivity ✓

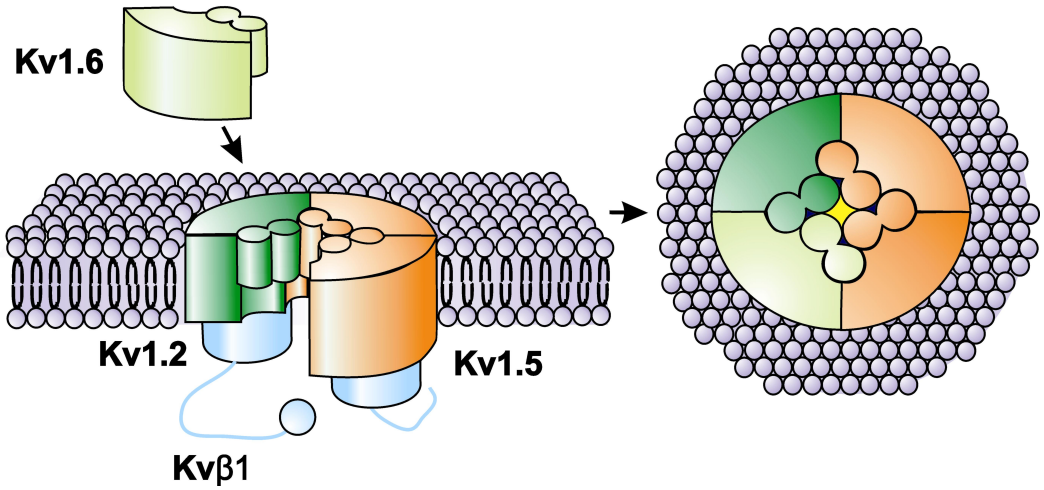
Activation time constants (C➔O) ✓

Deactivation time constants (O➔C) ✓

Inactivation time constants (O➔I) ✓

Other combinations of Kv subunits

Molecular composition of Kv1 channels in the mesenteric vasculature



- **Alteration of Kv1 channel expression in disease**
- **Molecular approaches to study Kv1 channel function**

Molecular:
- RT-PCR
- real-time PCR

Immunocytochemical:
- subunit-specific antibodies

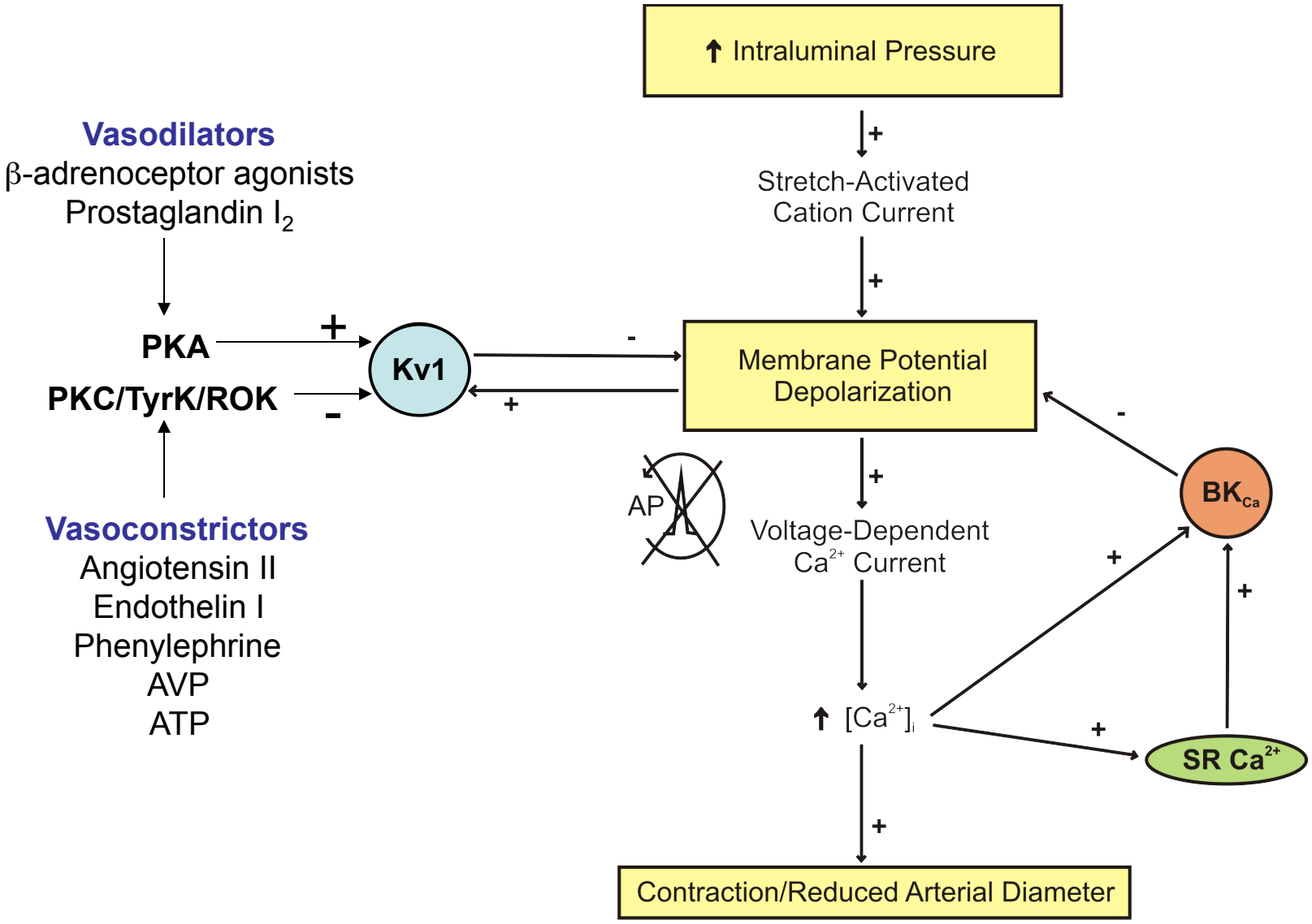
Pharmacological:
- dendrotoxins
- 4-AP

Biophysical:
- V-dependence
- inactivation rate
- deactivation rate

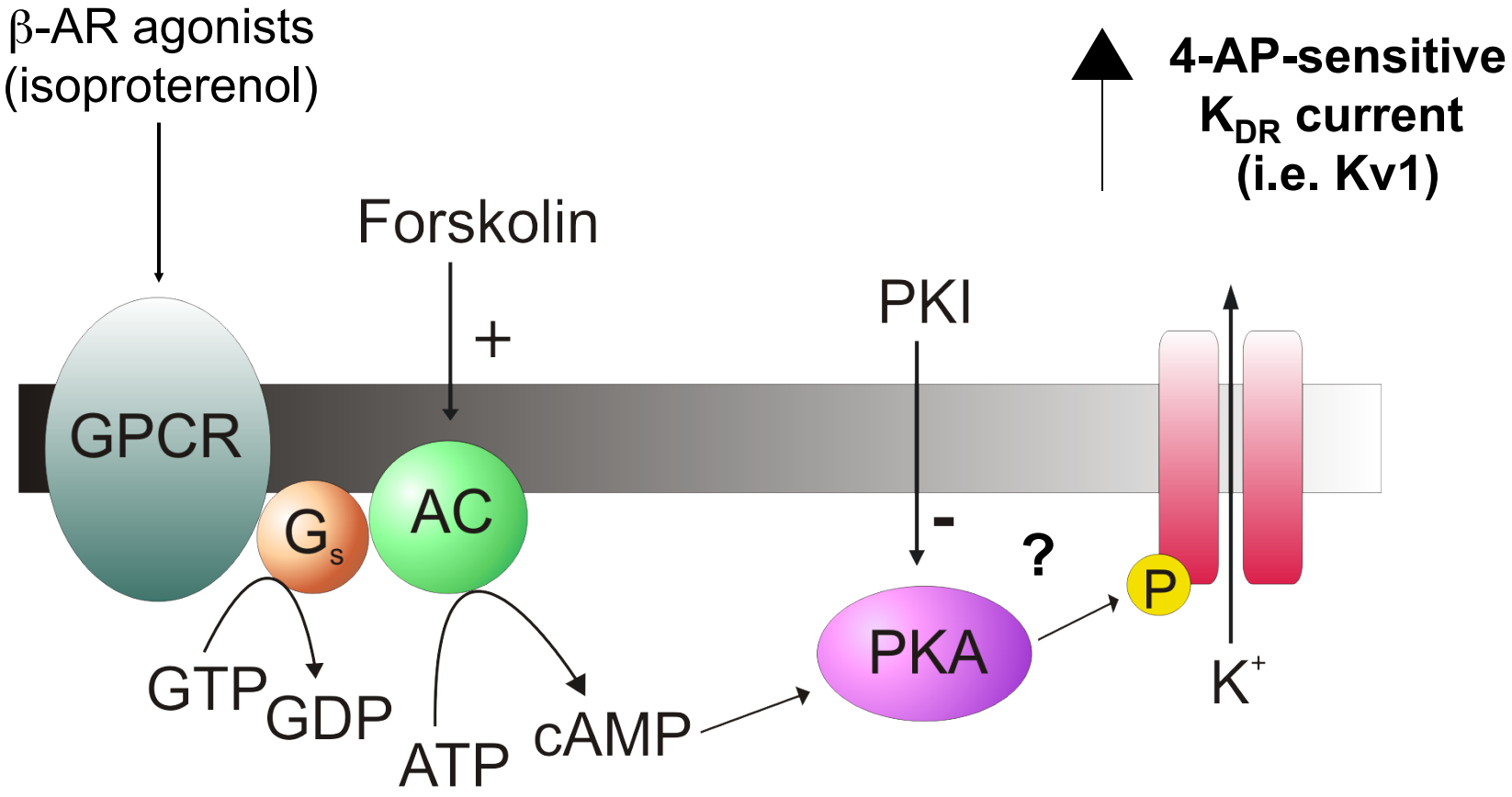
Part 2:

Molecular basis for regulation of vascular smooth muscle Kv1 channels by protein kinase A

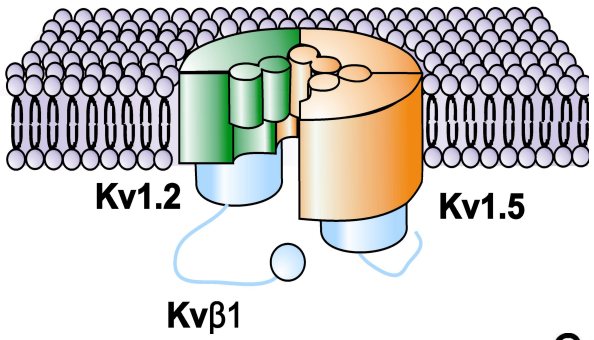
Modulation of Kv control of E_m by vasoactive agonists



Regulation of VSM Kv channels by PKA signalling pathways



Subunit-specific regulation of Kv1 channels by PKA



Rabbit Portal Vein

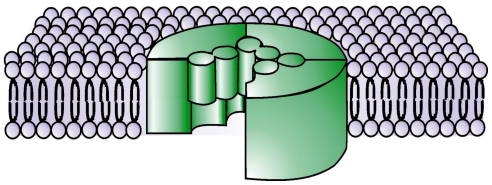
Coronary Artery

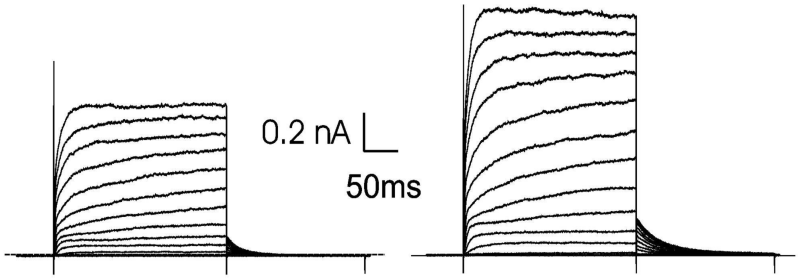
GI Smooth Muscle

Control

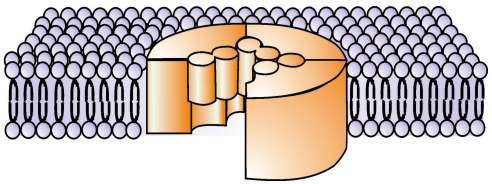
Forskolin (1 μM)

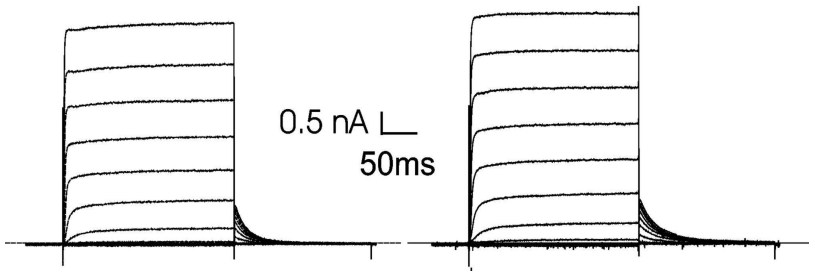
Kv1.2



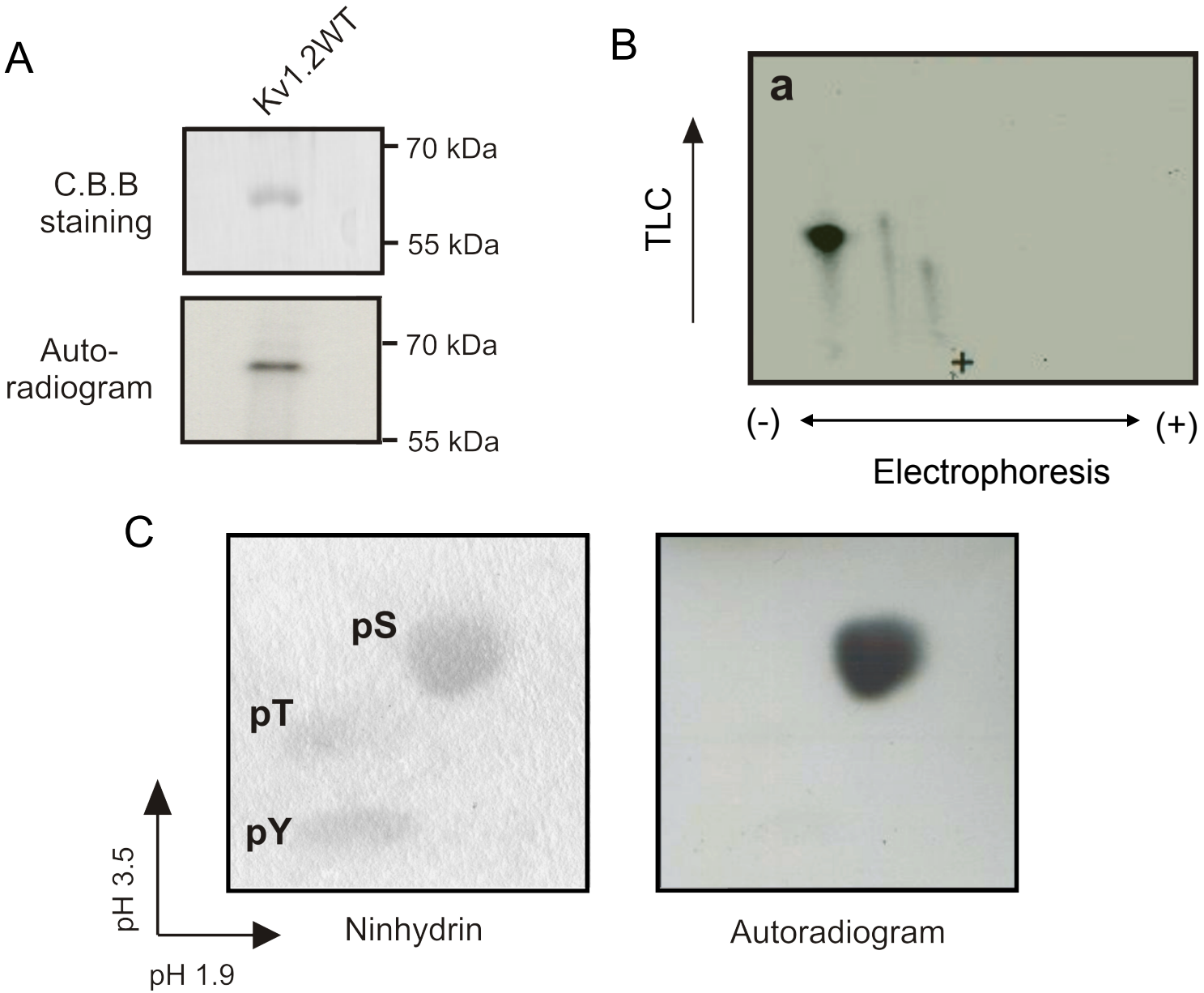


Kv1.5

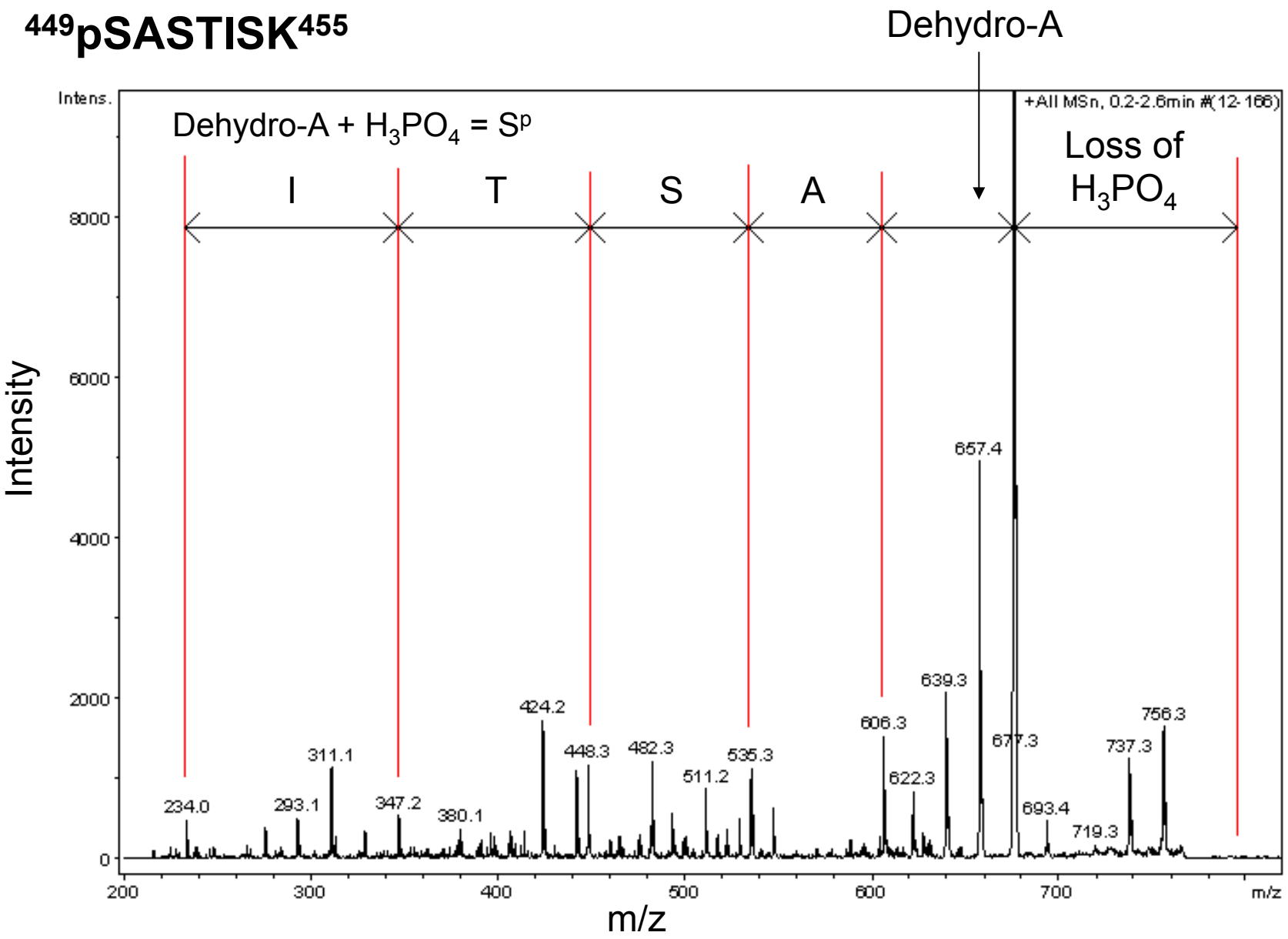




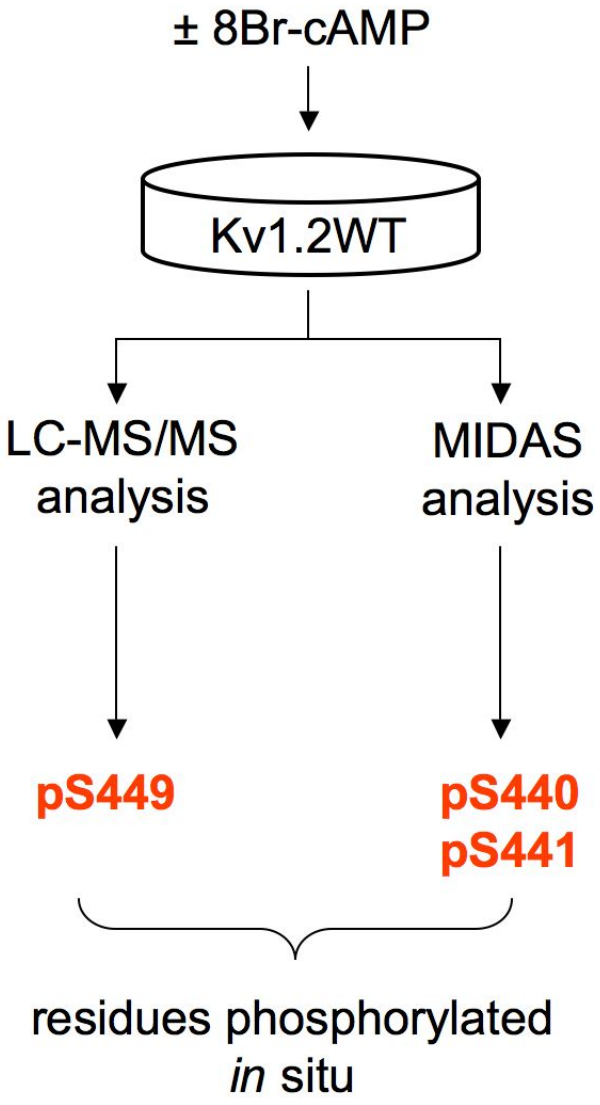
Kv1.2 is phosphorylated by PKA exclusively at serine

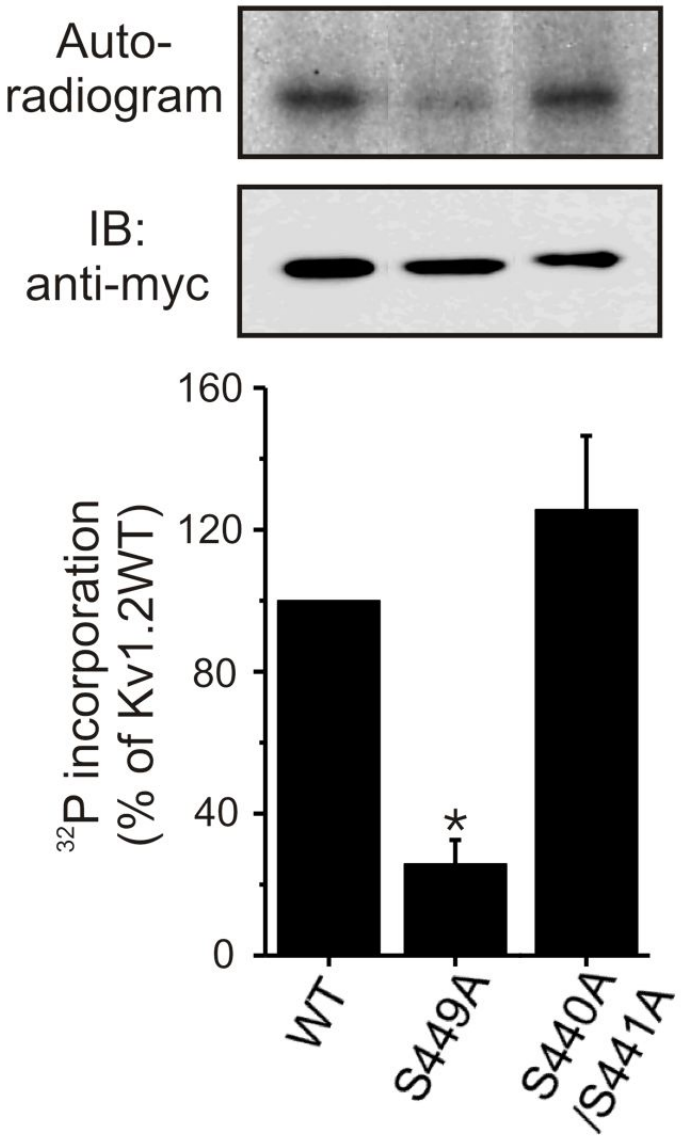


Kv1.2 is phosphorylated by PKA at serine-449 *in vitro*

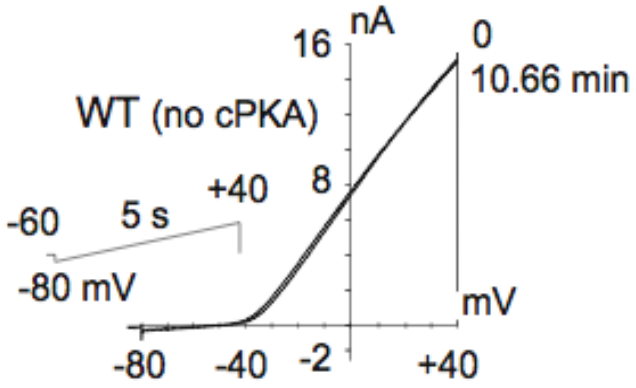


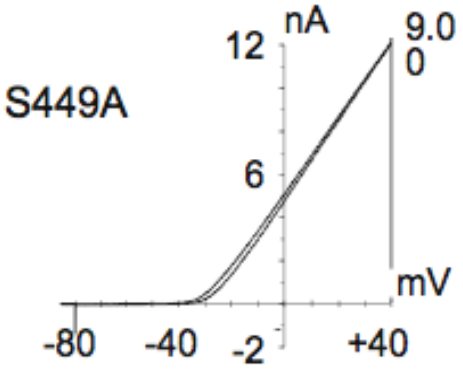
Kv1.2 is phosphorylated at S440, S441 and S449 *in situ* following 8Br-cAMP stimulation

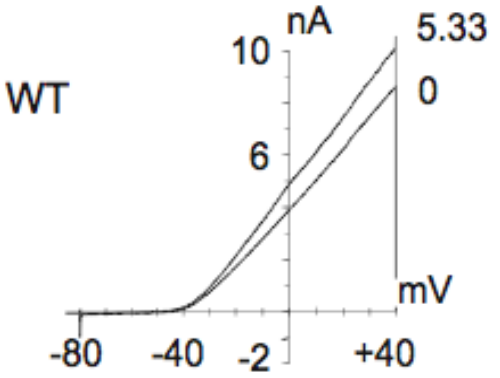


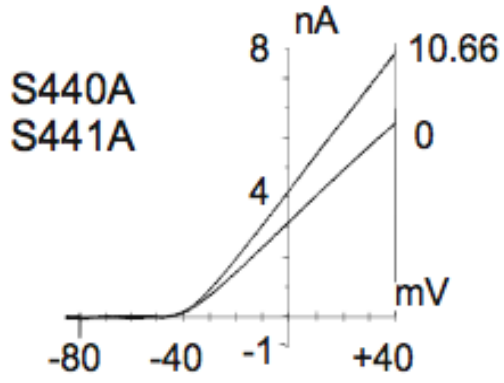


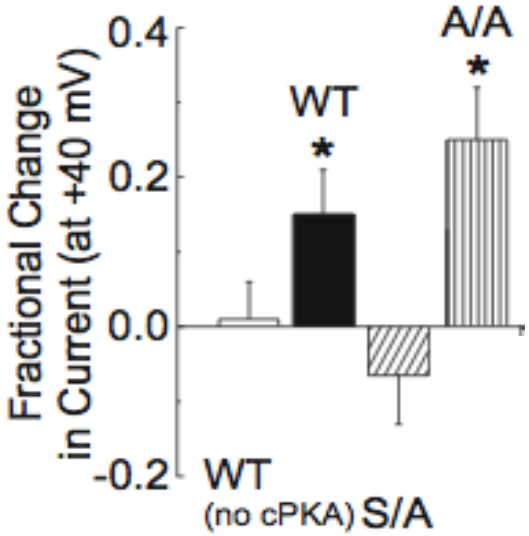
Phosphorylation at S449 (but not S440 and/or S441) is important for PKA-dependent changes in Kv1.2 current



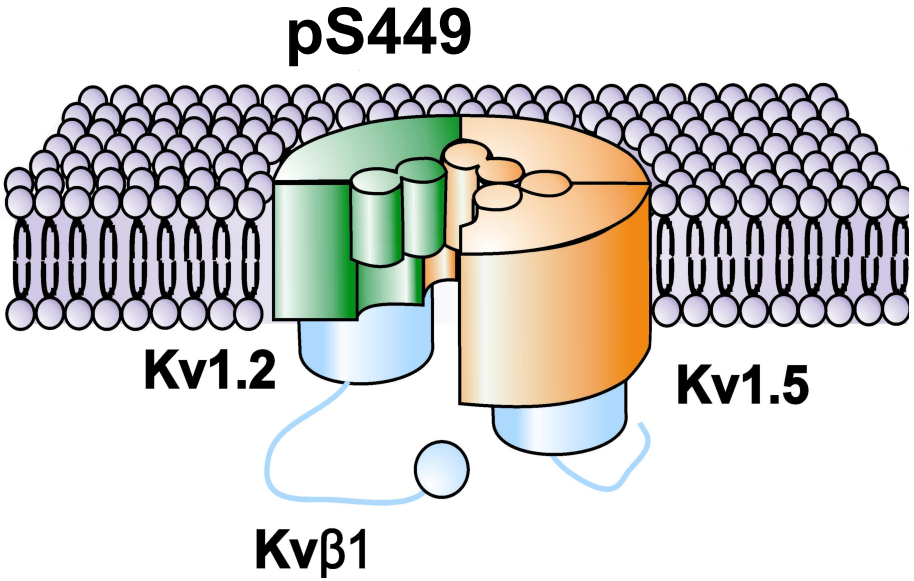








Future directions - characterization of S449 phosphorylation in VSM



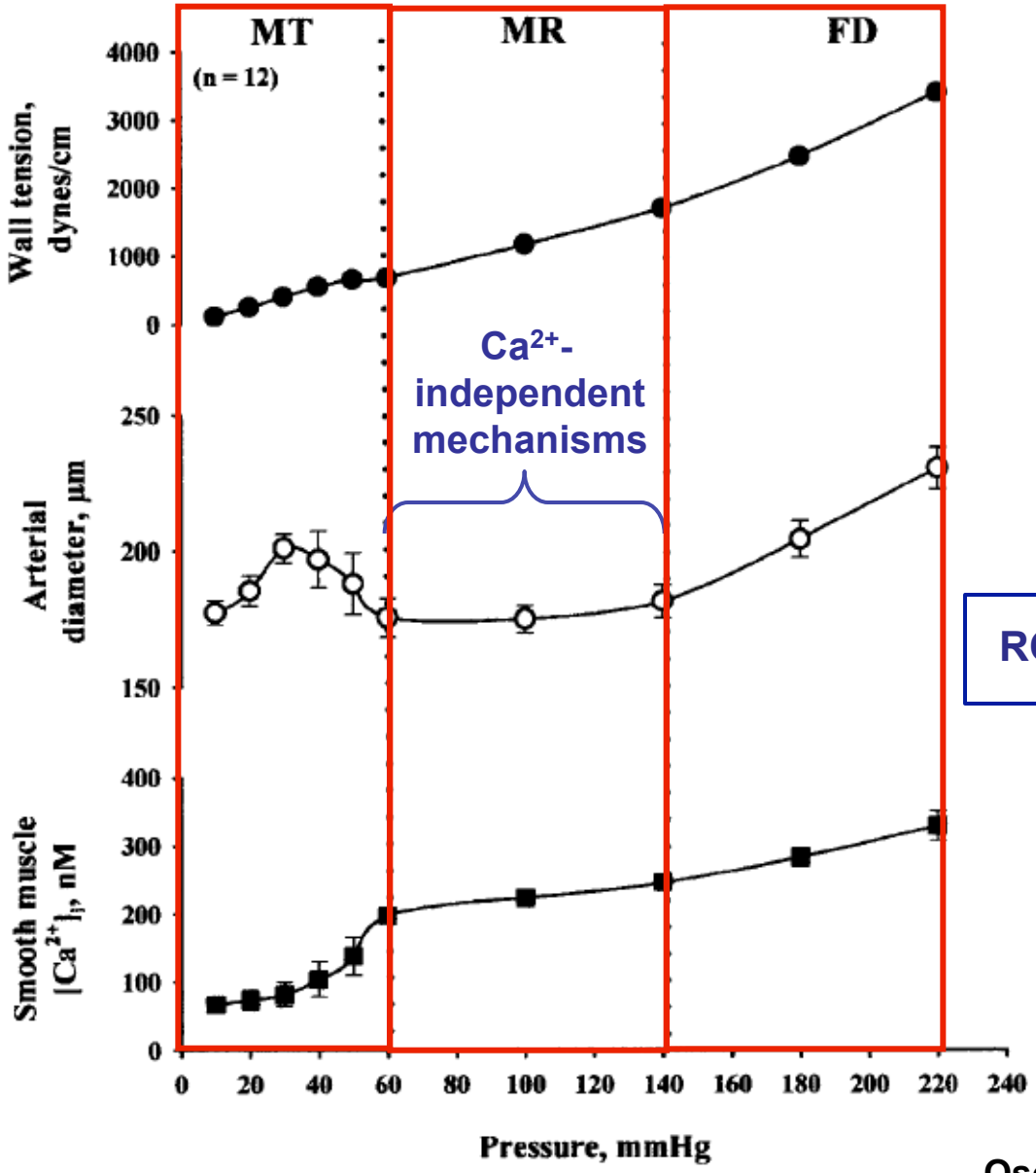
- Phosphorylation in heteromultimeric channels

- Analysis of Kv1.2 phosphorylation in VSM using phosphospecific antibodies (pS449)

Part 3:

Contribution of ROK-dependent Ca^{2+} sensitization pathways in myogenic control of arterial diameter in the cerebral vasculature

A three-phase model of the myogenic response



MT = myogenic tone

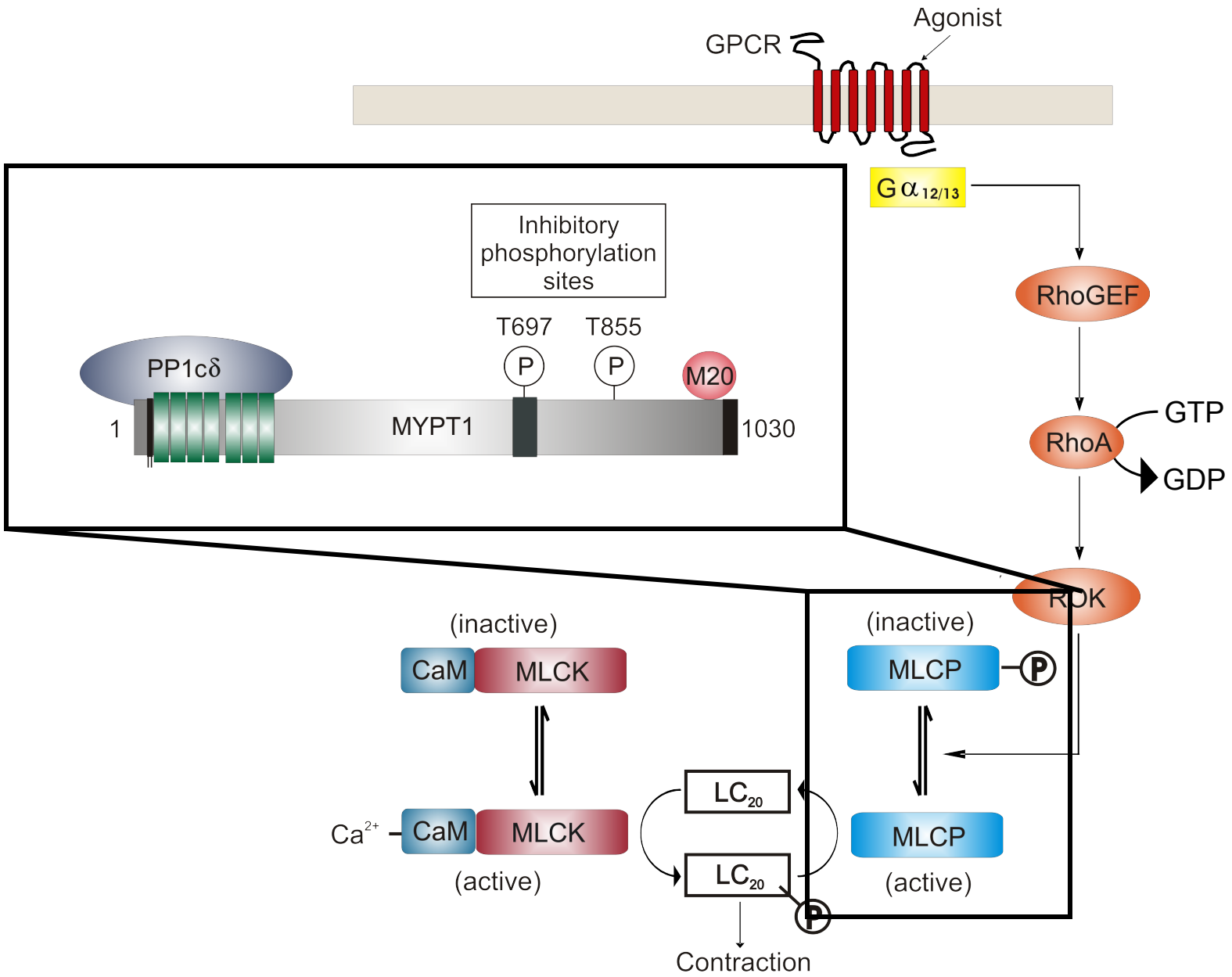
MR = myogenic reactivity

FD = forced dilation

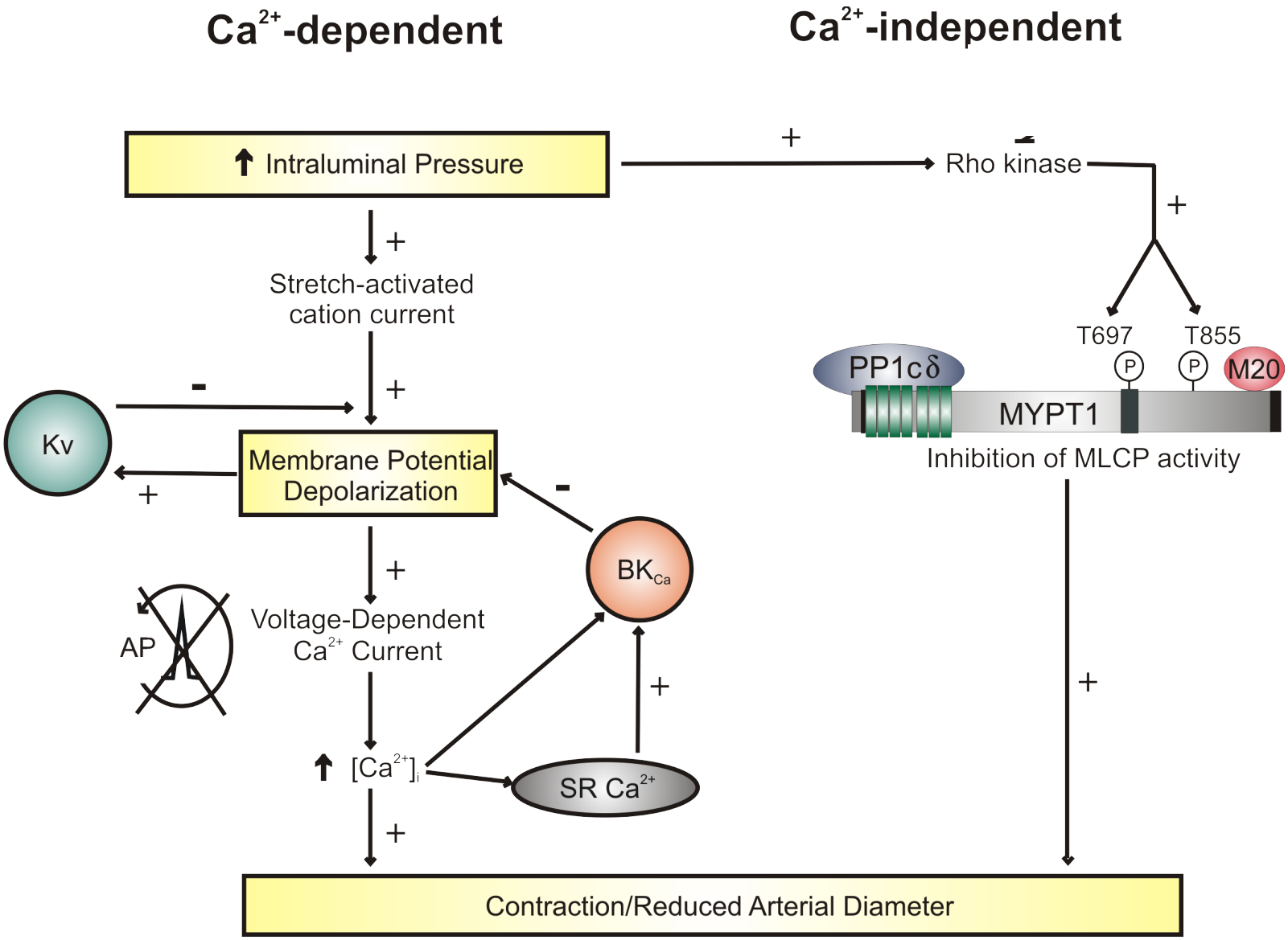
ROK-dependent Ca^{2+} sensitization

Osol *et al*. Am. J. Physiol. 282: 2260 (2002)

ROK-dependent Ca^{2+} sensitization in VSM



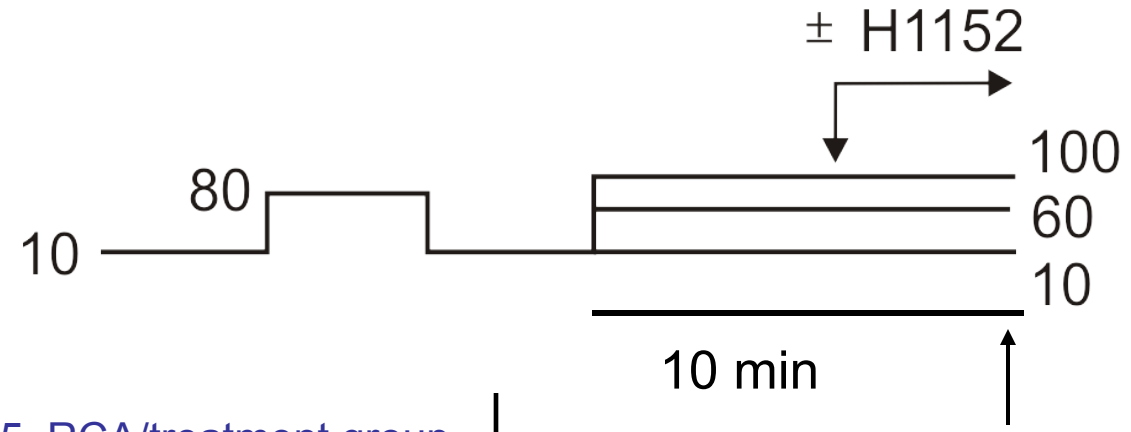
ROK-dependent Ca^{2+} sensitization and the myogenic response



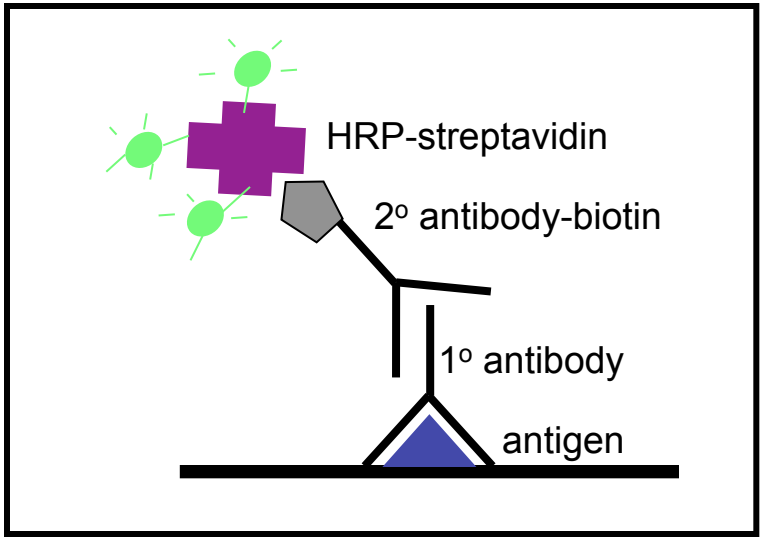
Tissue preparation for analysis of pMYPT1/LC_{20} in pressurized RCA

Rat cerebral artery
(Pressure arteriography)

0.5 - 1.0 mm long
0.1 - 0.2 mm in diameter
2-5 smooth muscle cells thick



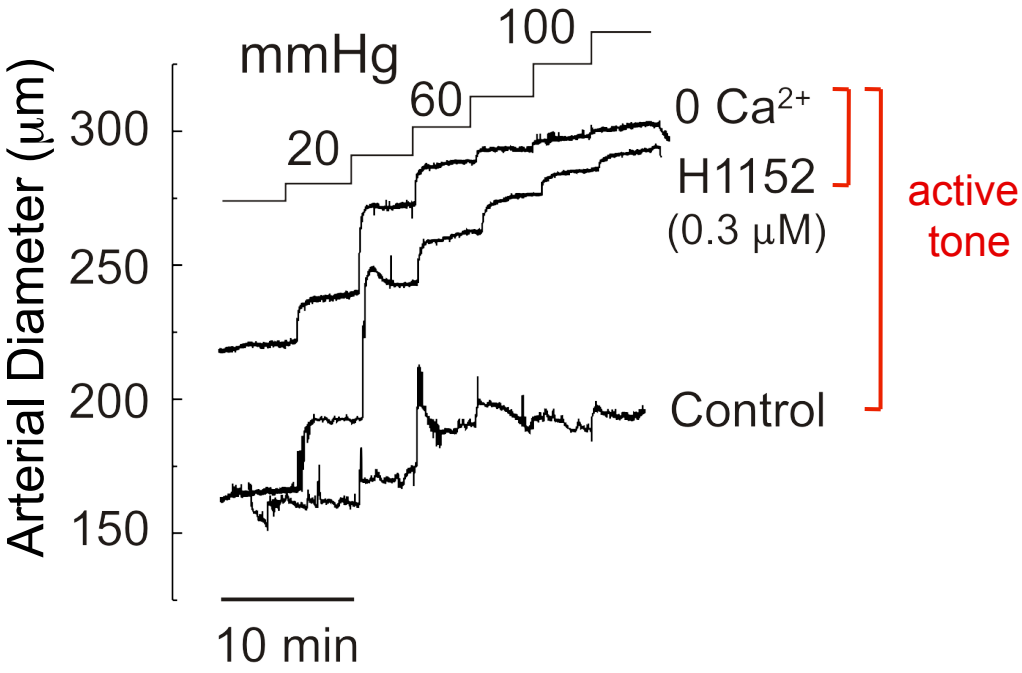
~5 RCA/treatment group
(i.e. 30 pressure myograph experiments/western blot!)

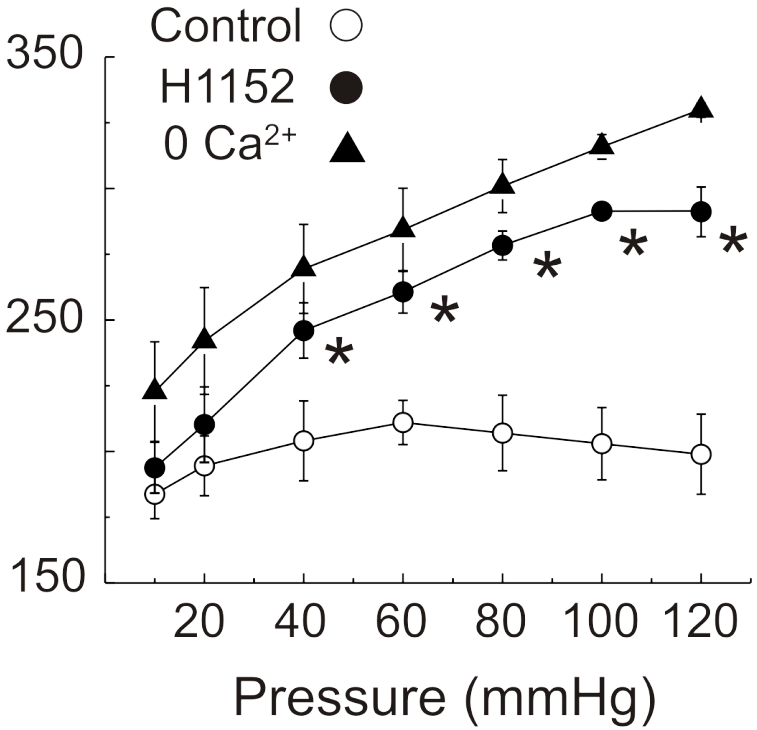


Western blot
anti-MYPT1-pT697
anti-MYPT1-pT855
anti-LC_{20}

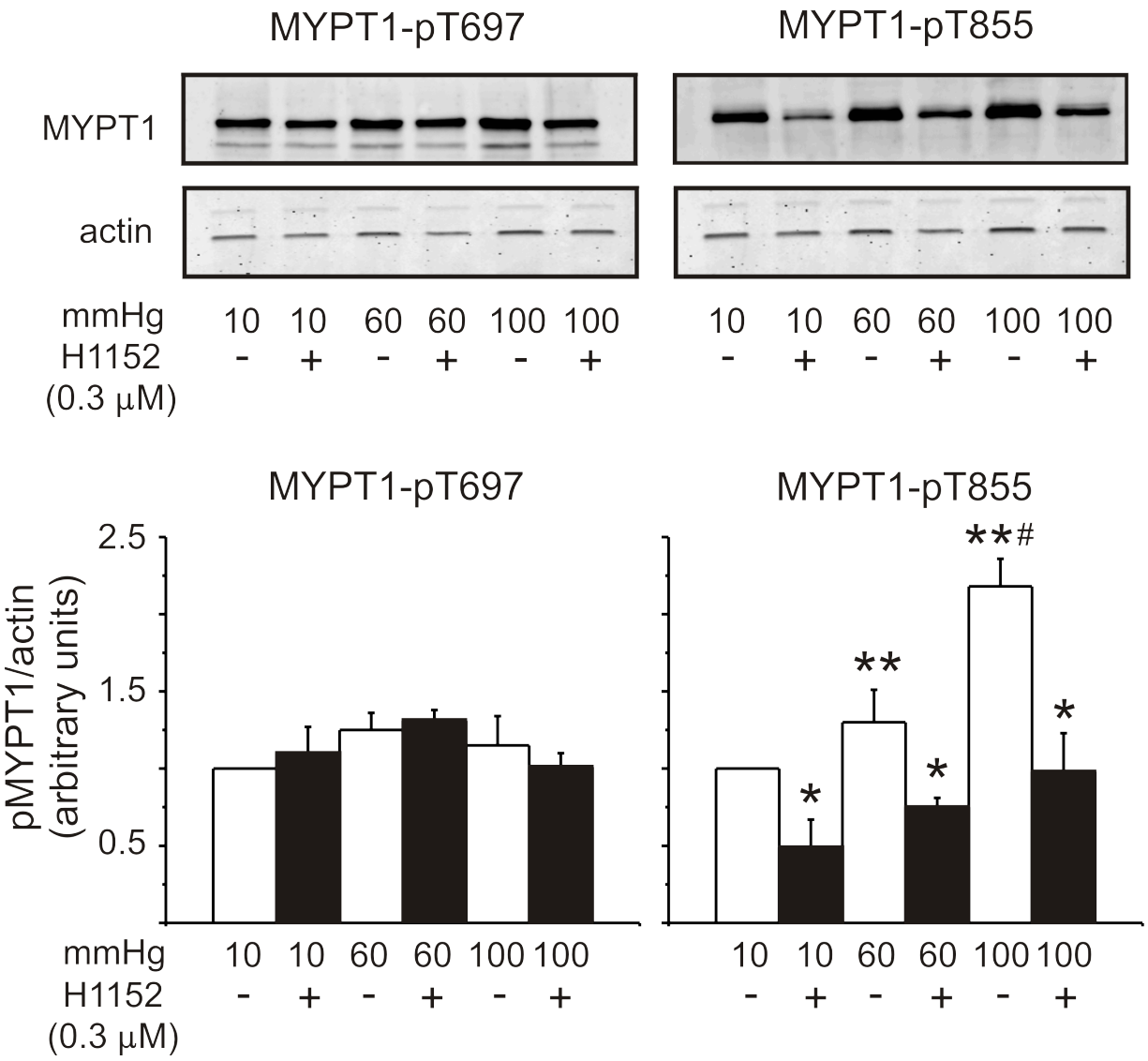
~ 10-fold increase in detection sensitivity

ROK inhibition impairs myogenic responsiveness

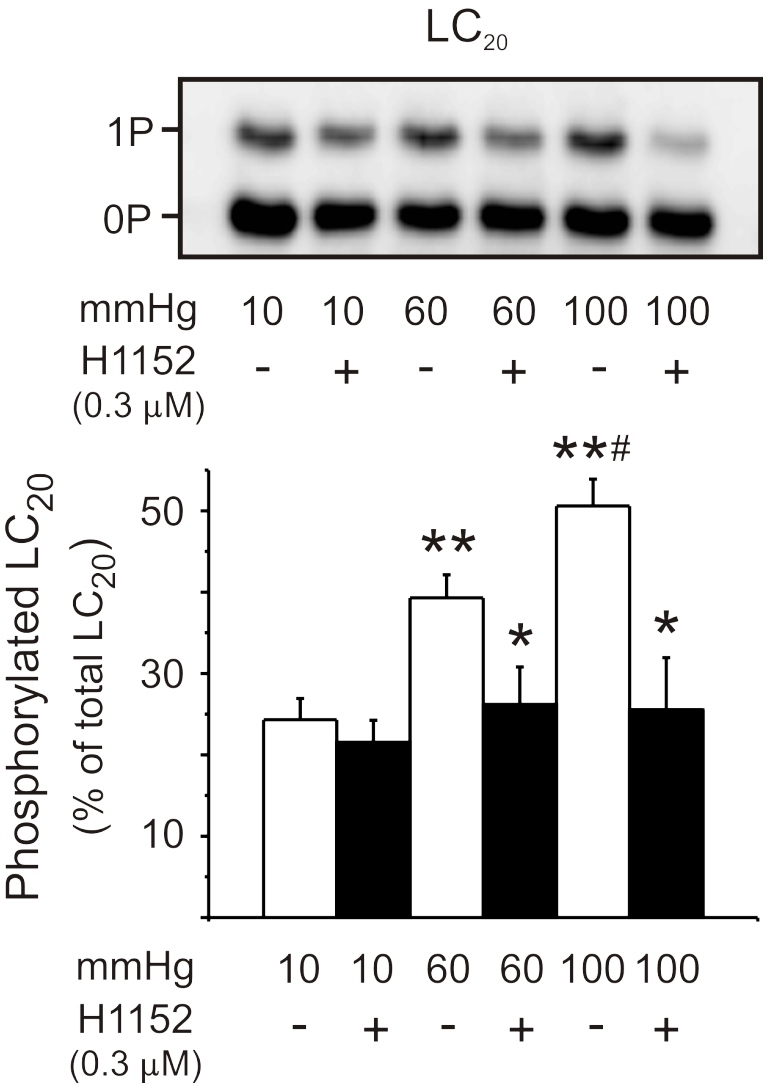




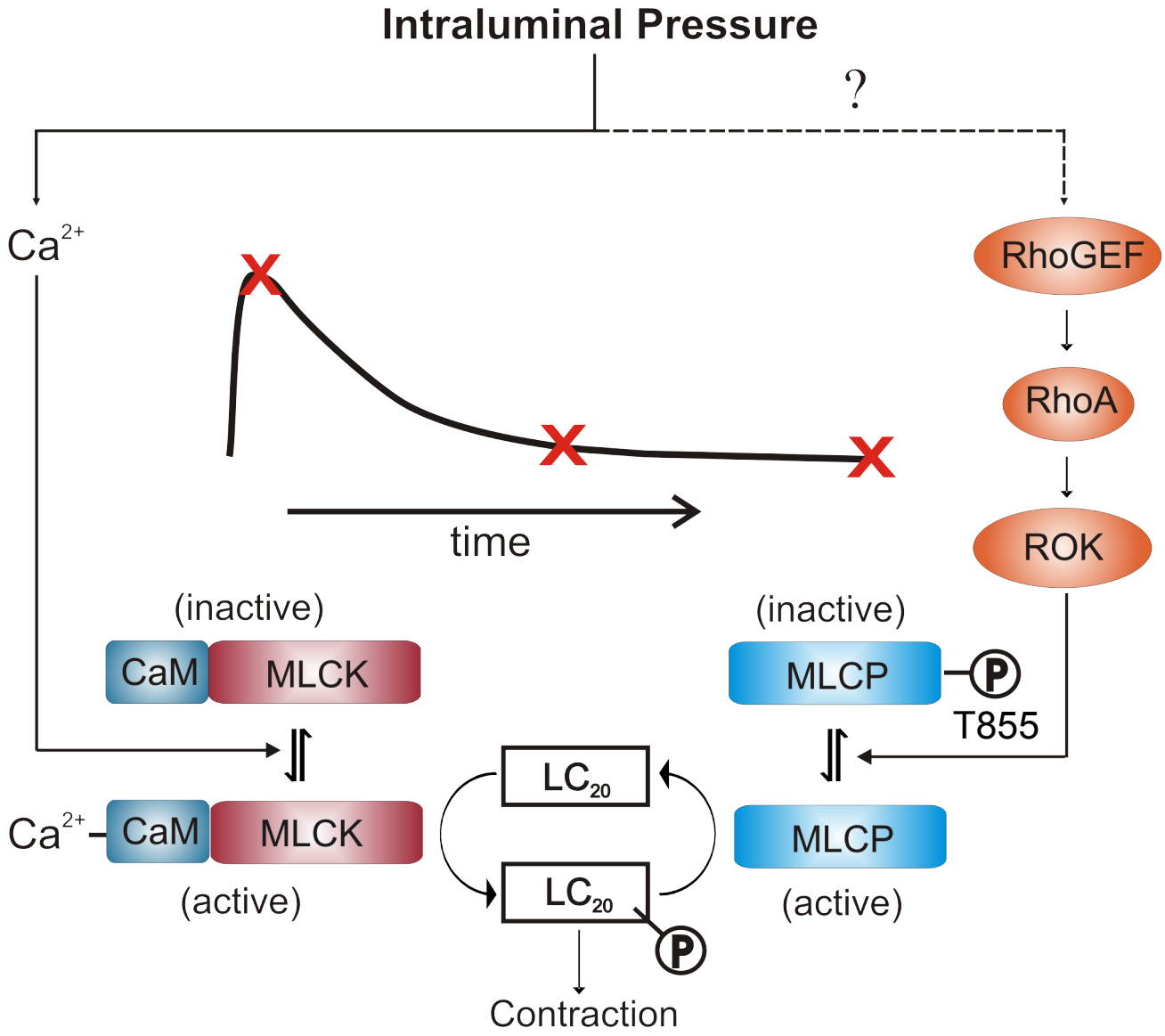
Elevation of intraluminal pressure evokes ROK-dependent MYPT1 phosphorylation at T855, but not T697

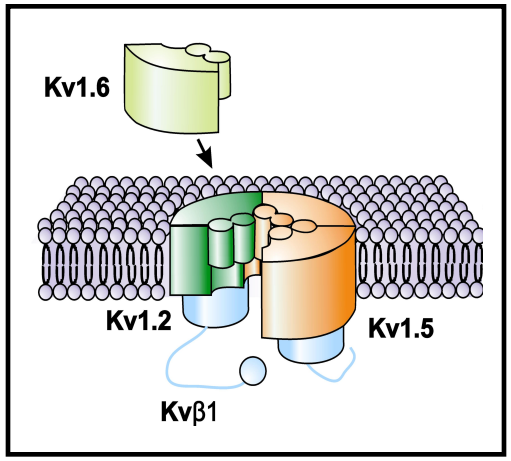


Elevation of intraluminal pressure evokes ROK-dependent LC_{20} phosphorylation



ROK-dependent Ca^{2+} sensitization contribute to myogenic control of arterial diameter





↑ Intraluminal Pressure

+

ROK

+

Stretch-activated
cation current

+

T855

P

M20

PP1cδ

MYPT1

Inhibition of MLCP activity

Vasodilators

-

PKA

+

Kv1

+

Membrane Potential
Depolarization

pS449

+

Voltage-dependent
Ca^{2+} current

+

MYPT1-pT855

10 10 60 60 100 100
- + - + - +

8Br-cAMP

WT

4 min

0

1 nA

50 ms

S449A

0

14

Auto-
radiogram

IB:
anti-myc

+

Contraction/↑ pLC_{20}