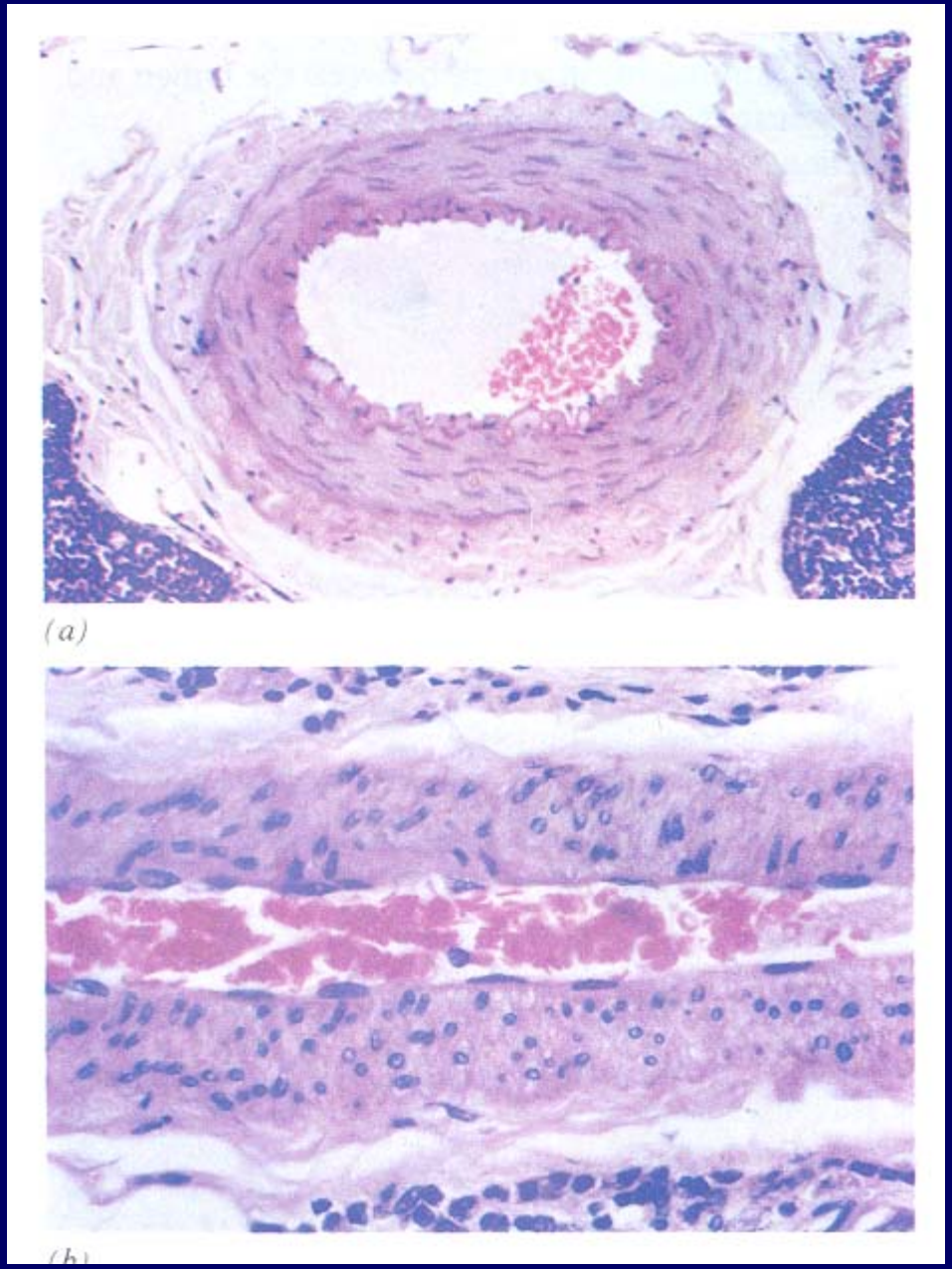


From: P.R. Wheater, H.G. Burkitt, V.G. Daniels, Functional Histology , 1979

Kv7 CHANNELS IN SMOOTH MUSCLE AS THERAPEUTIC TARGETS FOR VASCULAR AND AIRWAY DISEASES

Kenneth L. Byron, Ph.D
Professor of Pharmacology
Loyola University Chicago

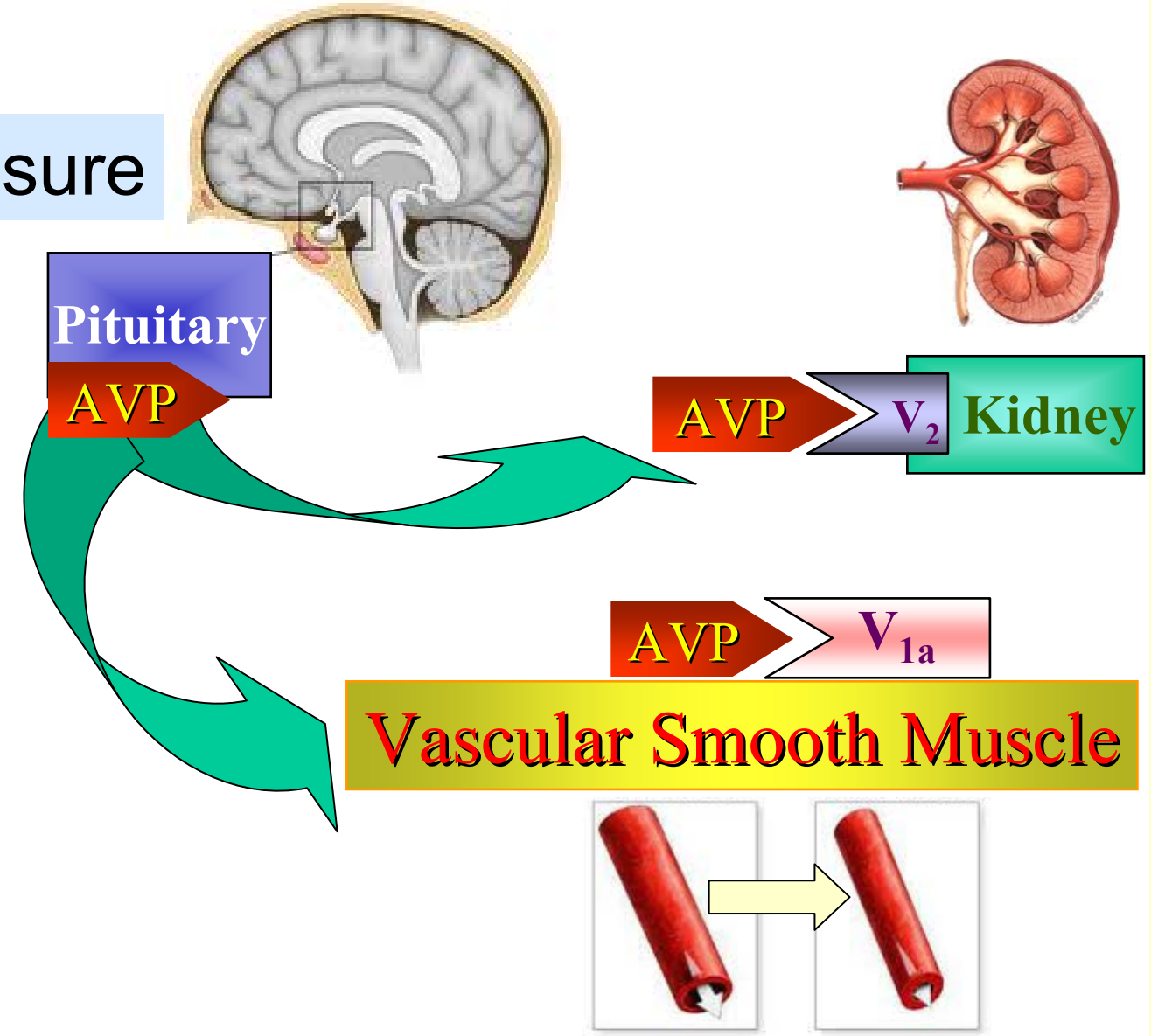
DISCLOSURE

- U.S. Patents issued:
  - Patent 8686017 "**Methods of using proteinacious channels to identify pharmaceutical treatments and risks, and treatments resulting therefrom**"
  - Patent 20,140,155,368 "**Combination pharmaceuticals and methods thereof using proteinacious channels as treatments for medical conditions**"

Arginine-Vasopressin (AVP): A pituitary hormone that regulates water balance and blood pressure

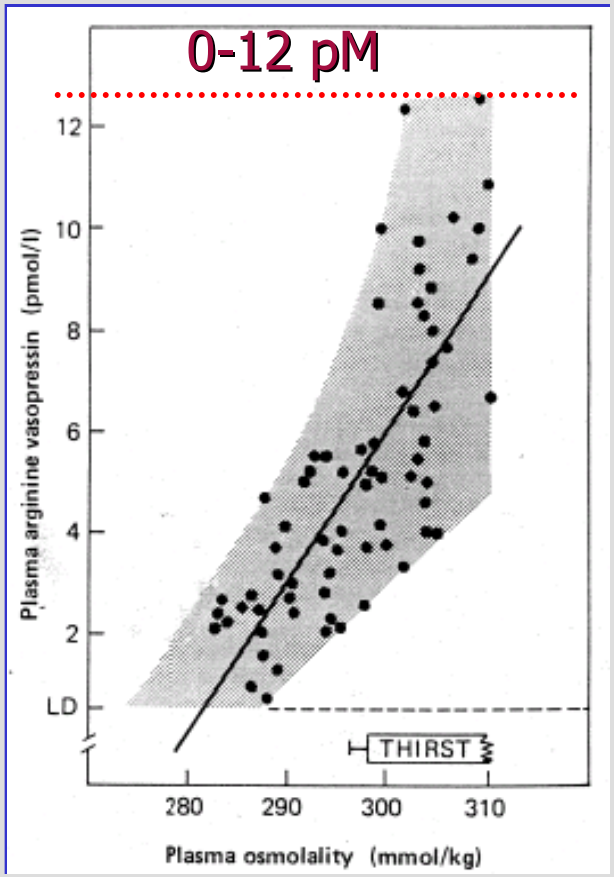
↓ Blood Pressure

↑ Plasma Osmolality

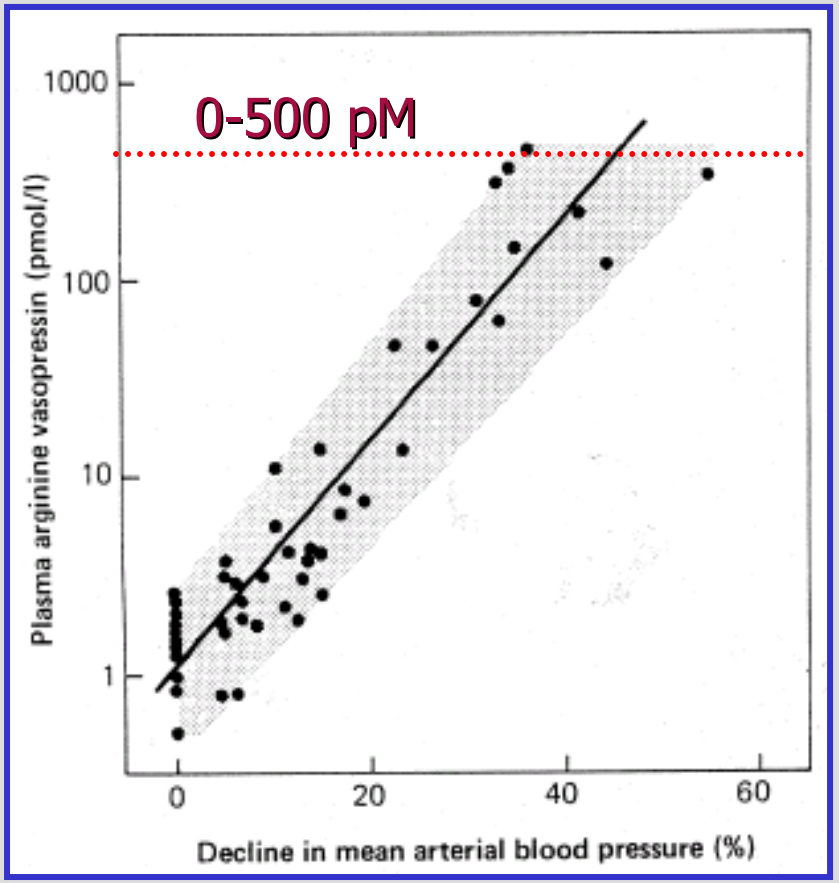


Plasma AVP Concentrations

Change in Osmolarity

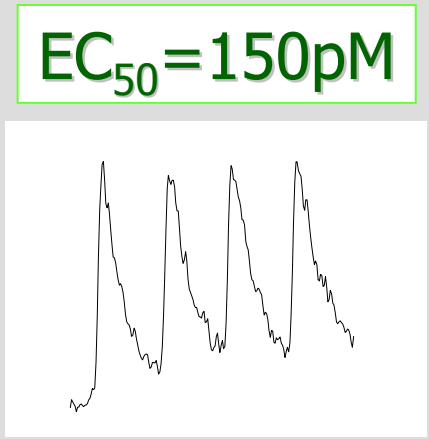


Change in Blood Pressure

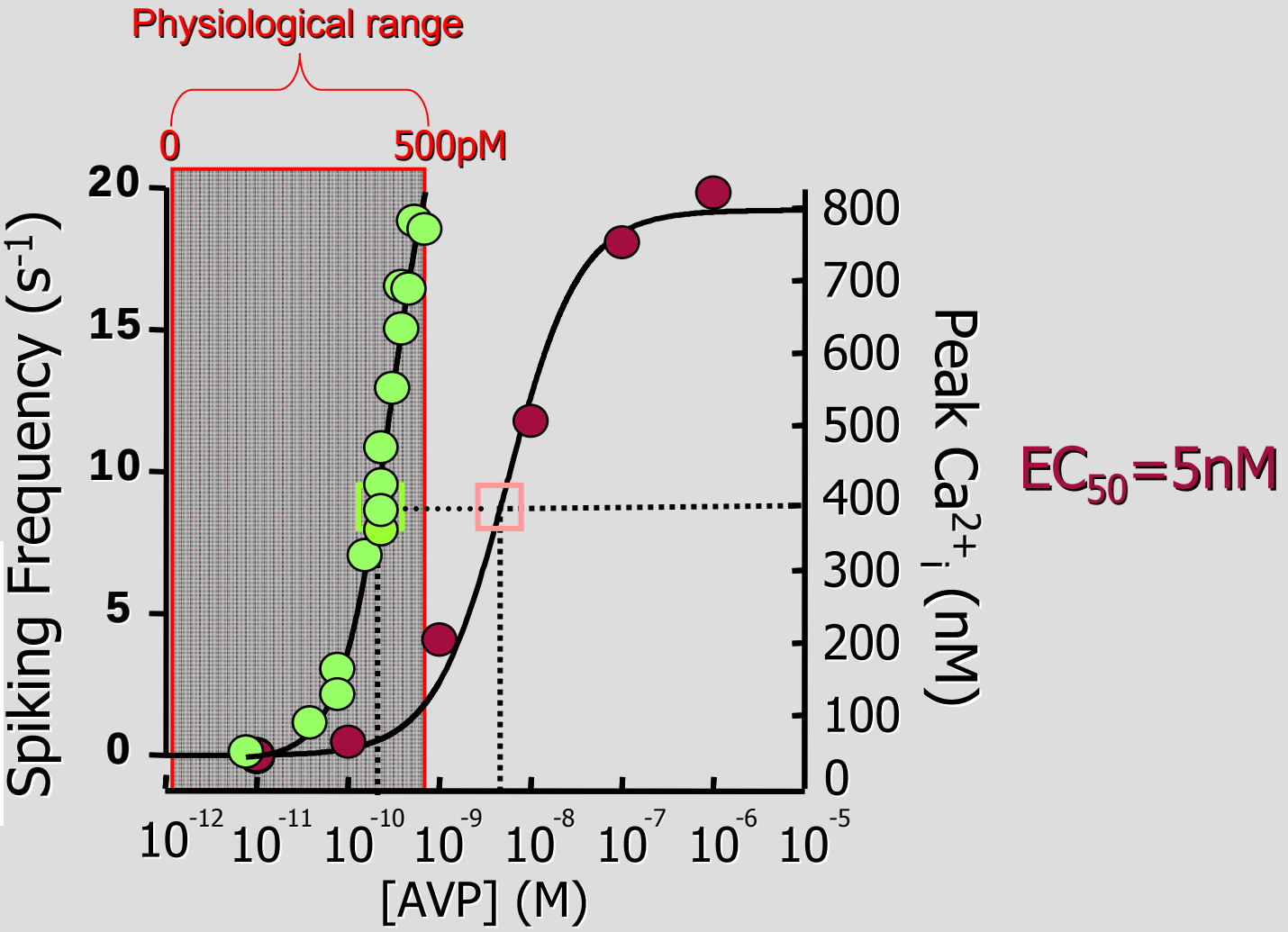


Baylis PH, Clin.Endocrinol.Metabol.1983

AVP Concentration-dependent Ca^{2+} Signaling in Vascular Smooth Muscle Cells (A7r5 cells)

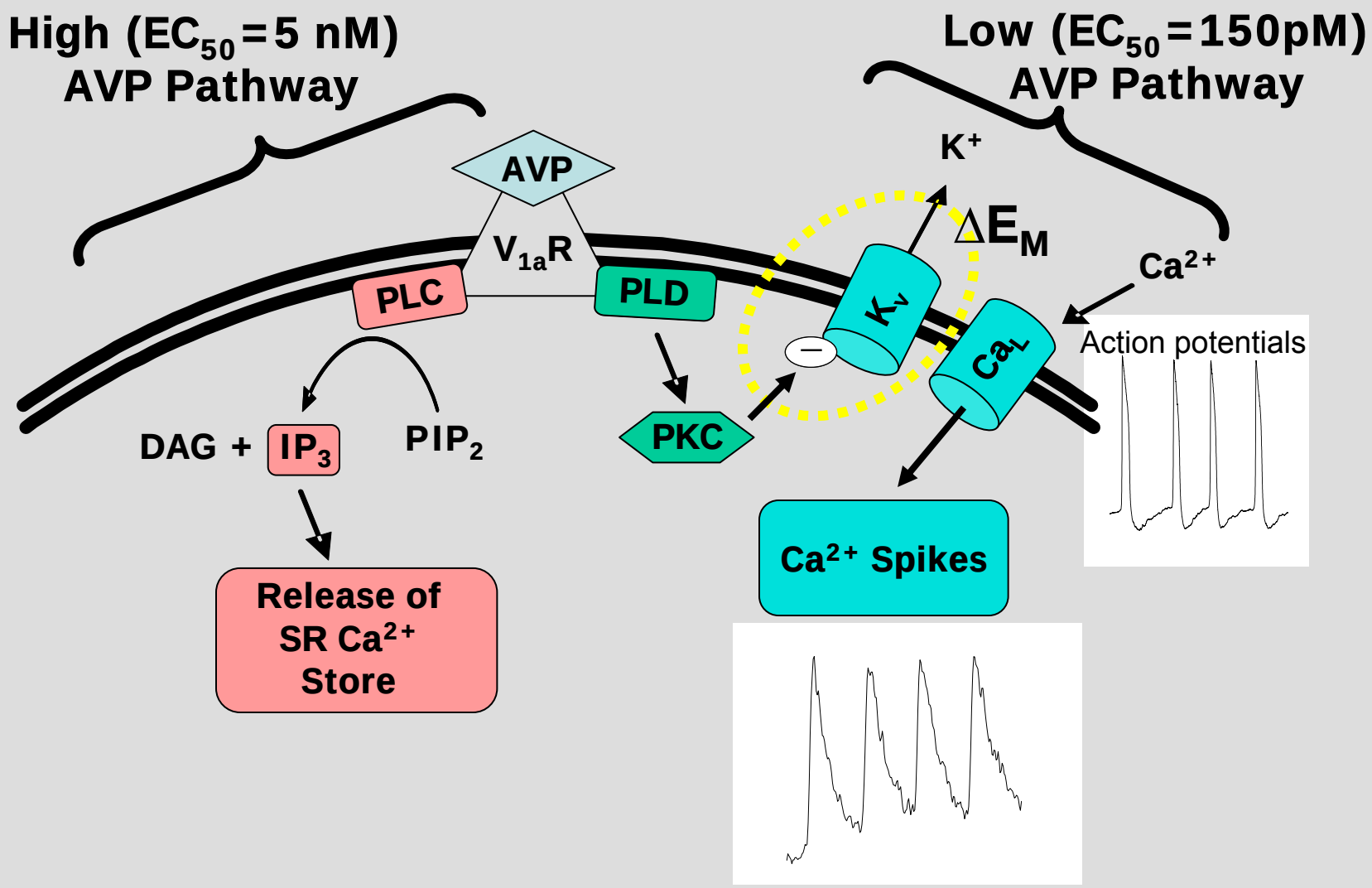


EC_{50}=150pM

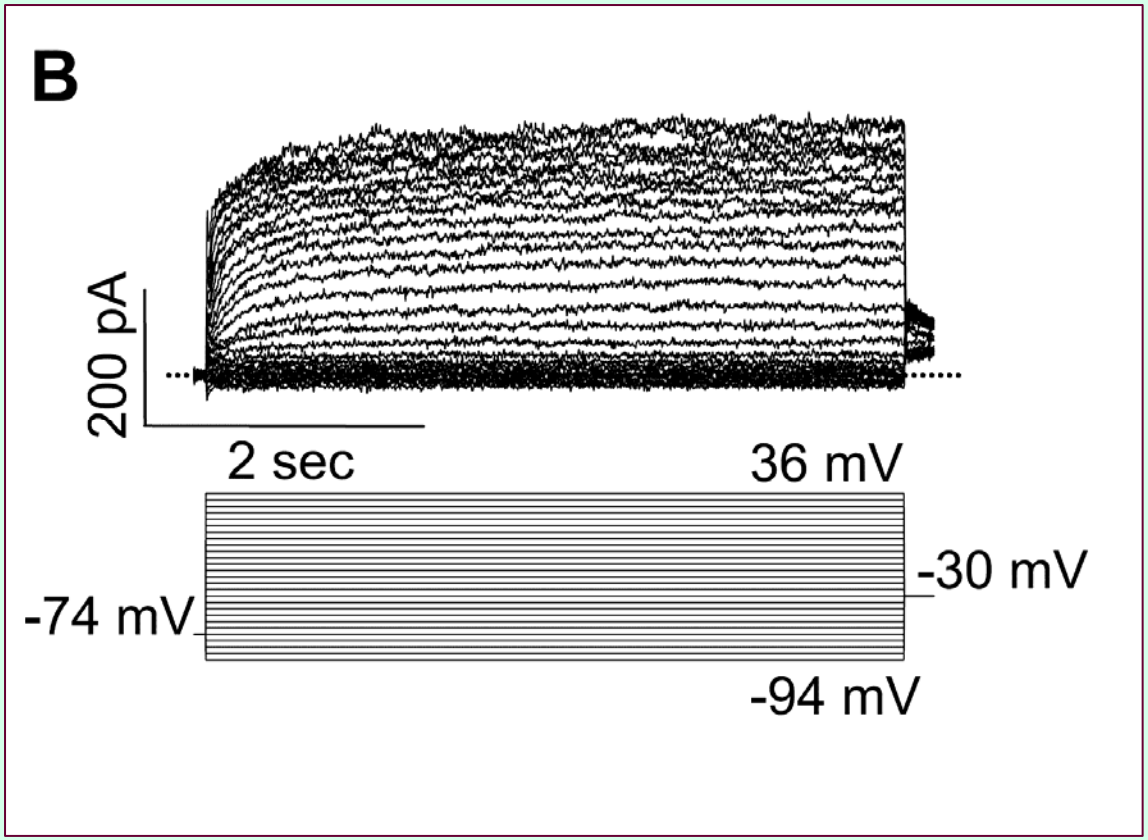


EC_{50}=5nM

Novel AVP signaling pathway identified in A7r5 rat aortic smooth muscle cell line



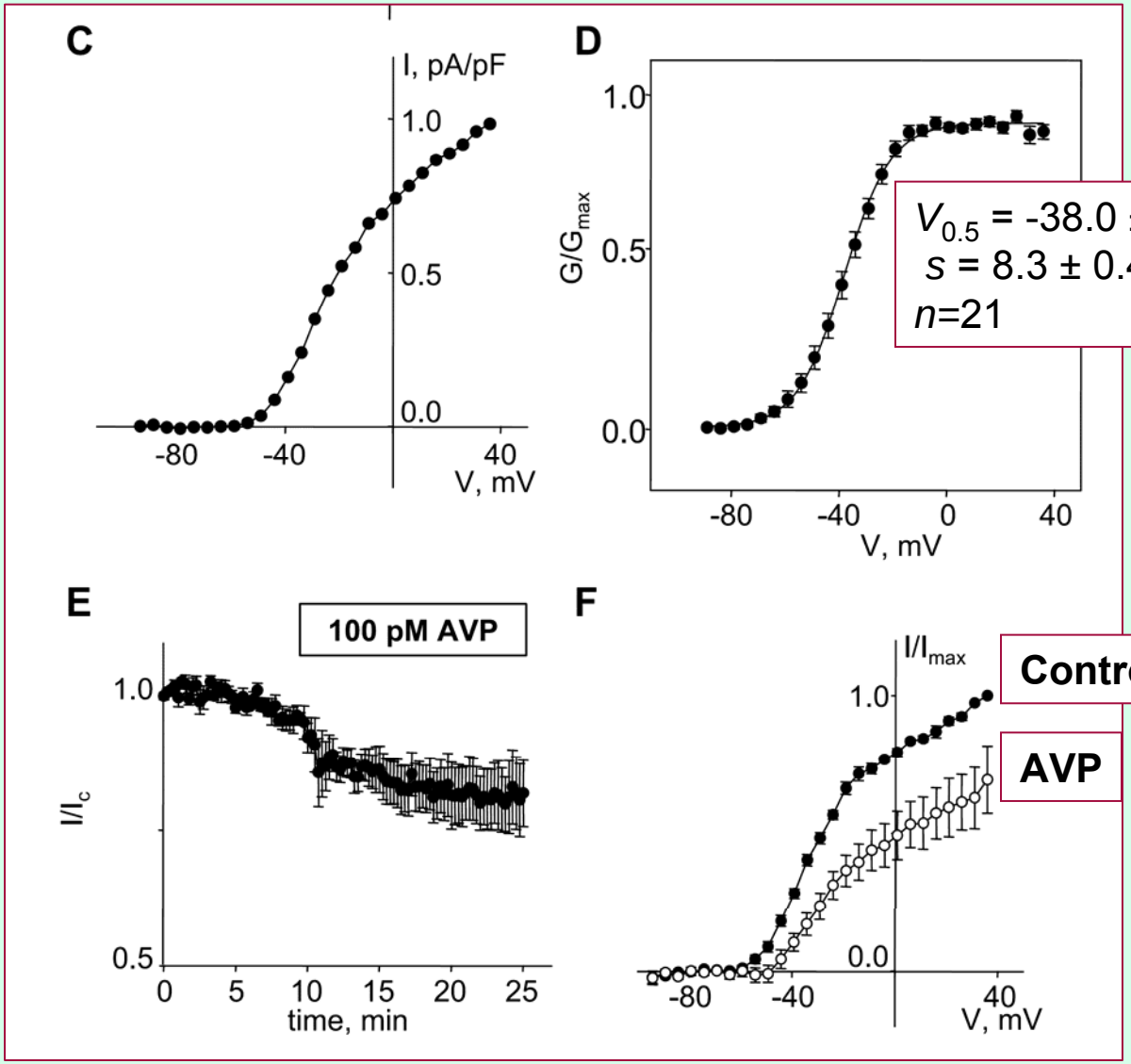
Isolated K_v currents are non-inactivating.



Dr. Lioubov Brueggemann

Brueggemann *et al.* Am J Physiol Heart Circ Physiol 292:1352-1363, 2007

Isolated K_v currents activate at very negative voltages and are suppressed by AVP



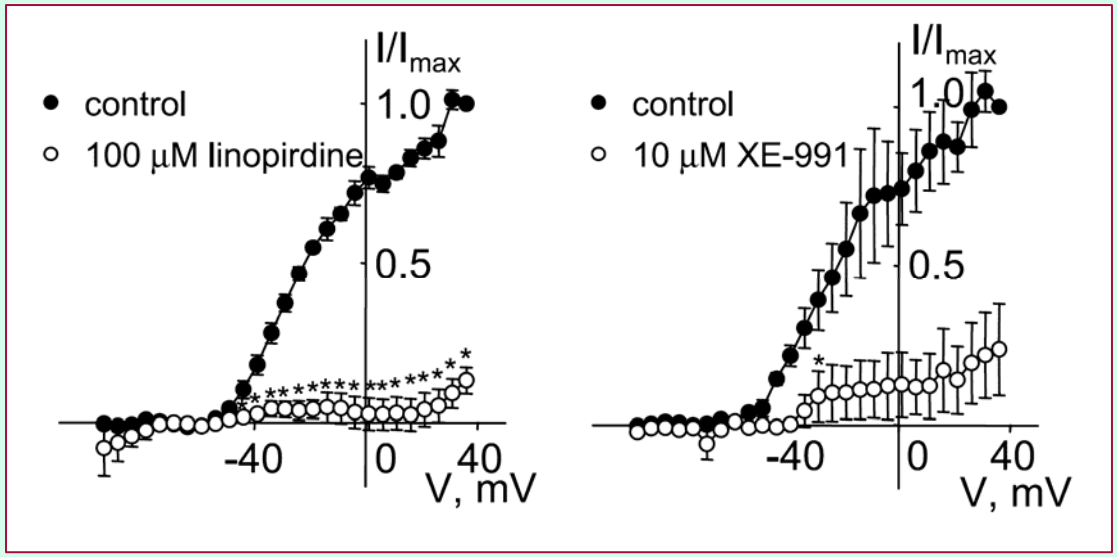
Brueggemann *et al.* Am J Physiol Heart Circ Physiol 292:1352-1363, 2007

AVP-sensitive K_v currents in A7r5 cells have characteristics of neuronal "M currents"

1. Non-inactivating delayed rectifier K^+ currents that activate over a relatively negative voltage range.

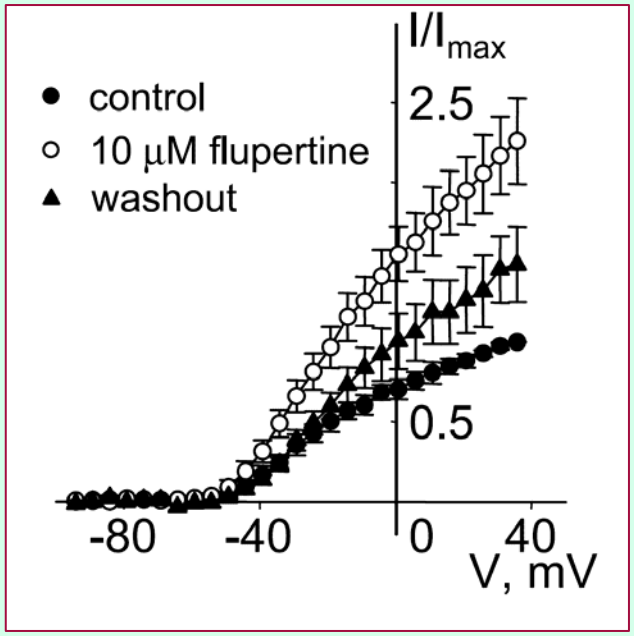
2. Inhibited in response to activation of G protein-coupled receptors, resulting in increased electrical excitability.

Are the vascular smooth muscle currents mediated by KCNQ (Kv7) channels?



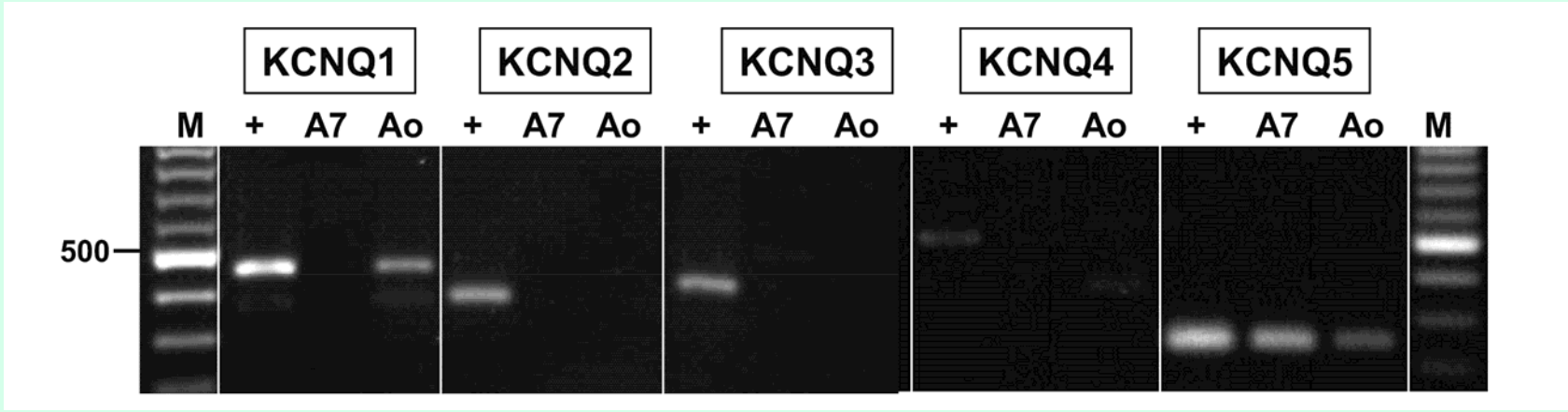
K_v currents are inhibited by selective KCNQ channel blockers linopirdine & XE991

and reversibly activated by selective KCNQ channel activator flupirtine



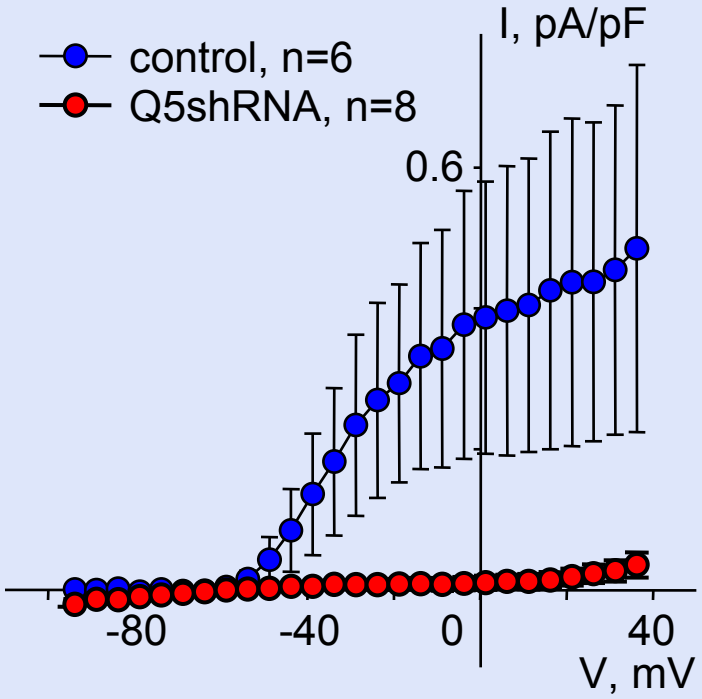
Brueggemann *et al.* Am J Physiol Heart Circ Physiol 292:1352-1363, 2007

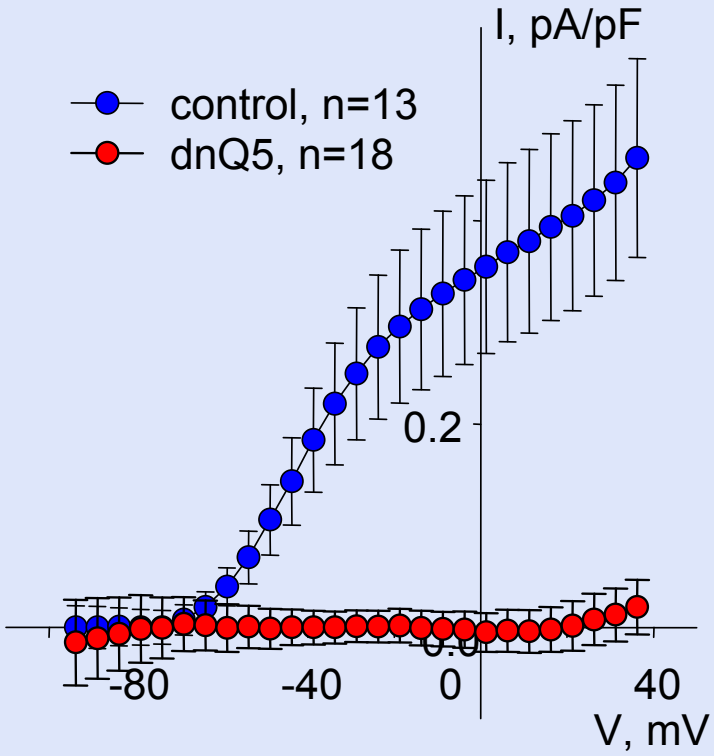
KCNQ5 is expressed in A7r5 cells.



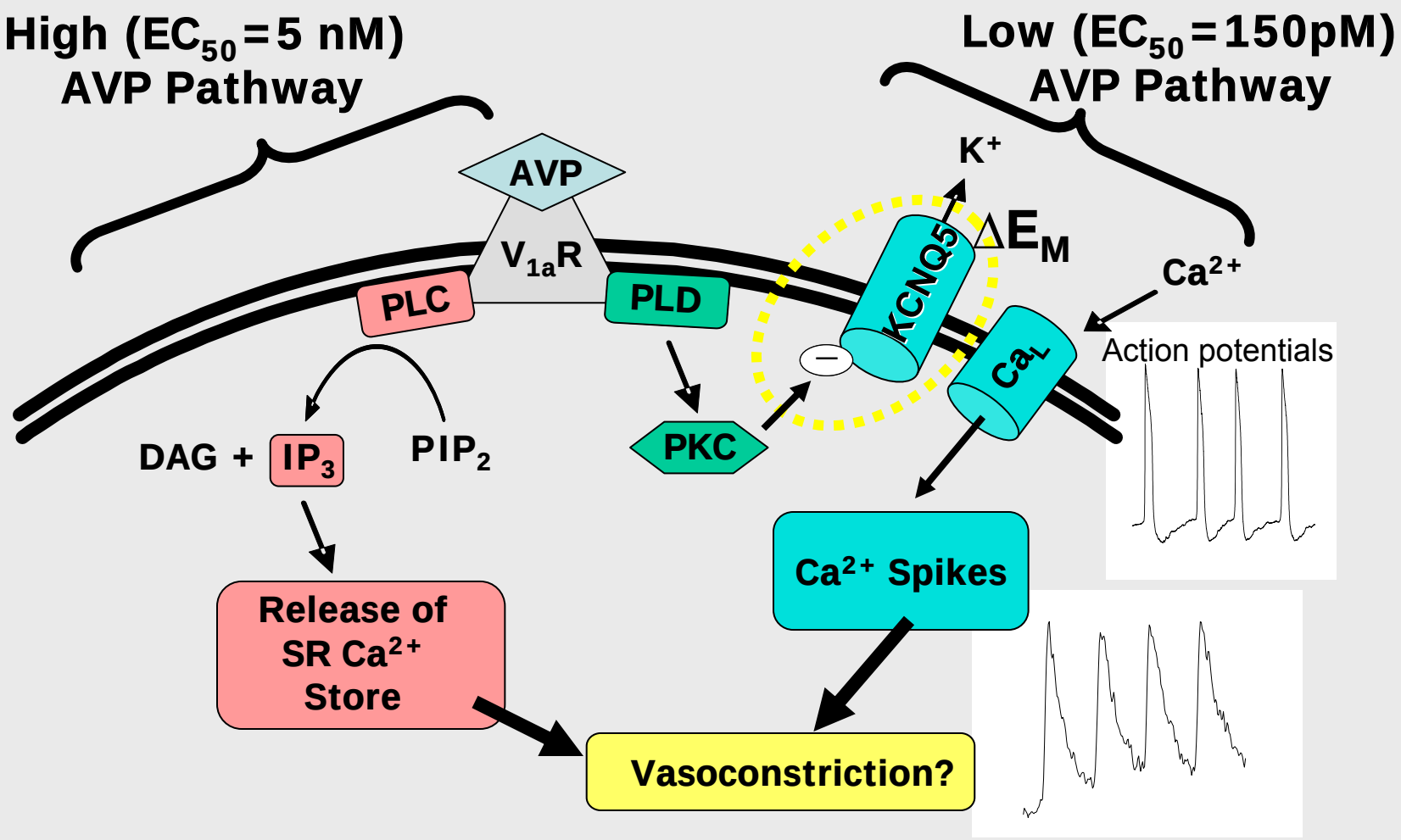
Brueggemann *et al.* Am J Physiol Heart Circ Physiol 292:1352-1363, 2007

Knocking down KCNQ5 expression or function abolishes the AVP-sensitive KCNQ currents.

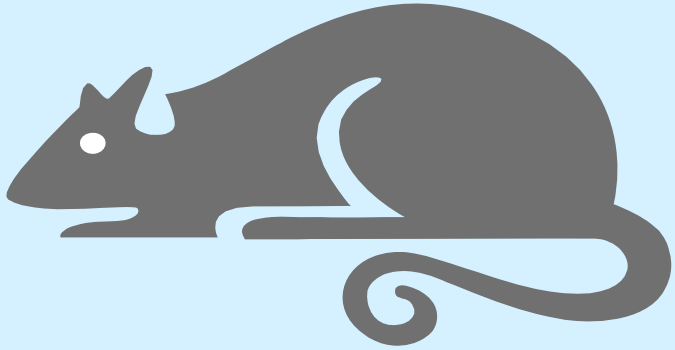




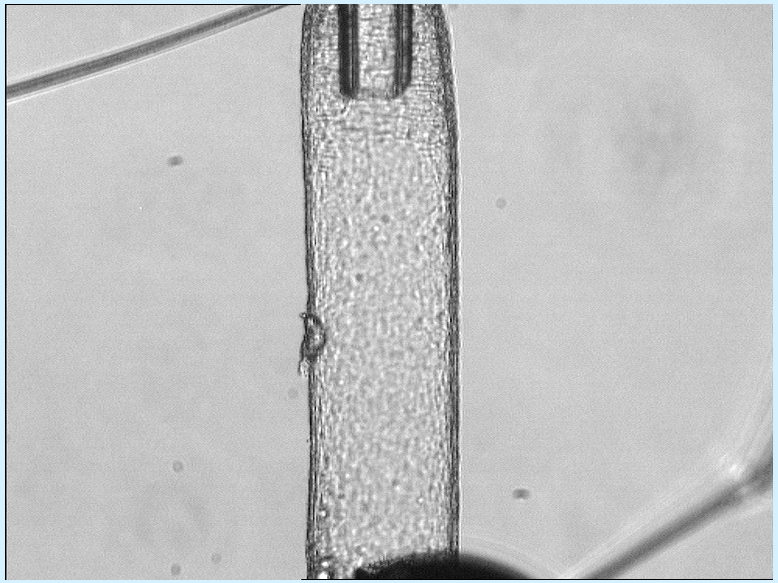
Novel AVP signaling pathway identified in A7r5 rat aortic smooth muscle cell line



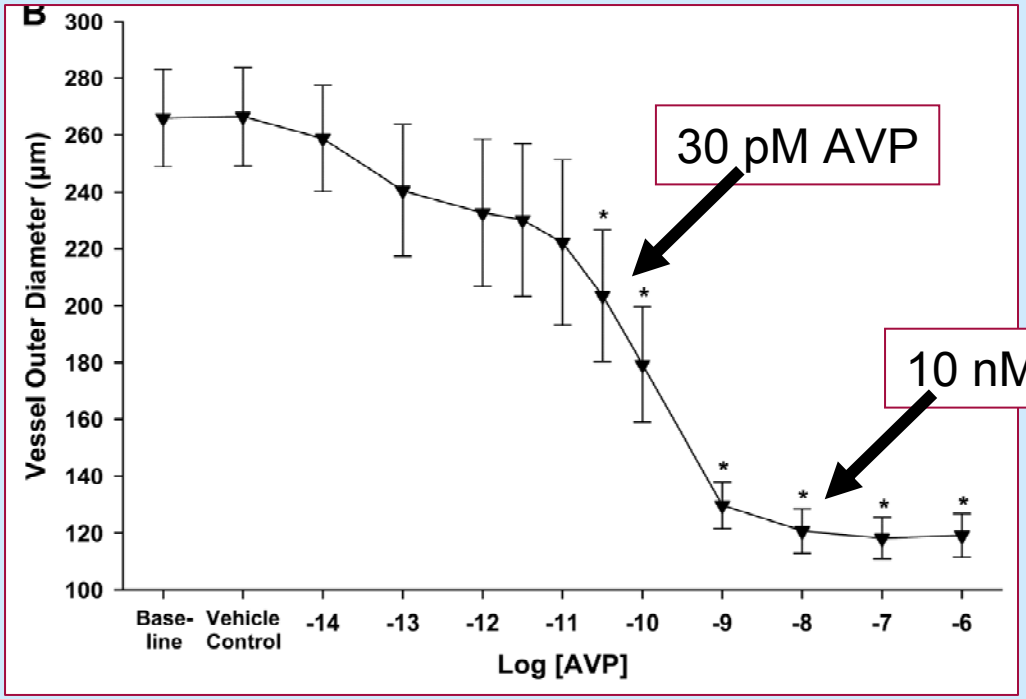
Vasoconstrictor responses measured in pressurized rat mesenteric arteries

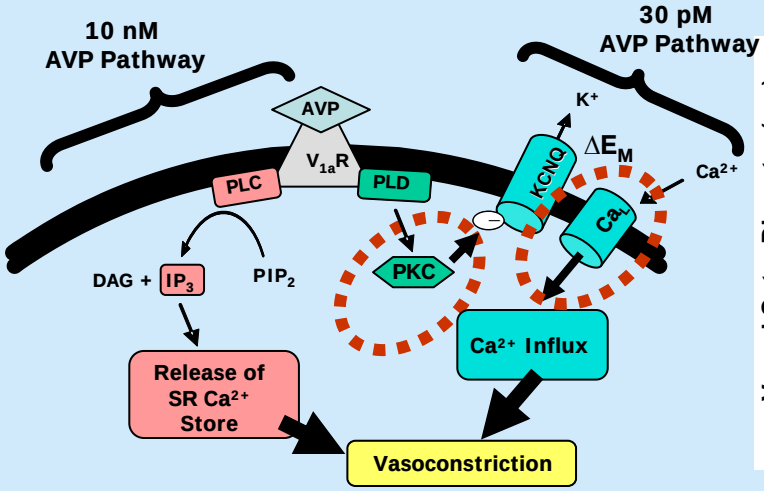


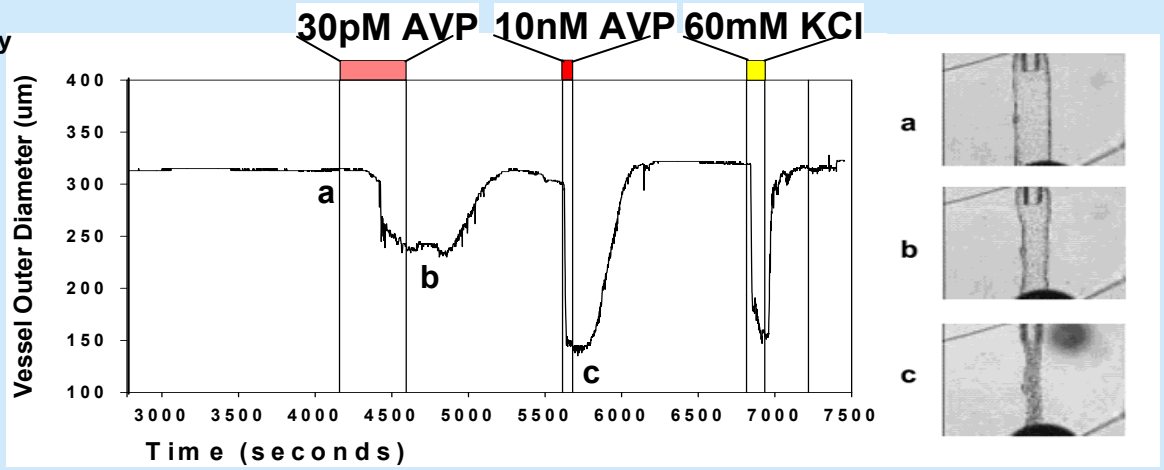
Outer diameters ~ 300-400 µm



AVP concentration-dependent constriction of rat mesenteric arteries

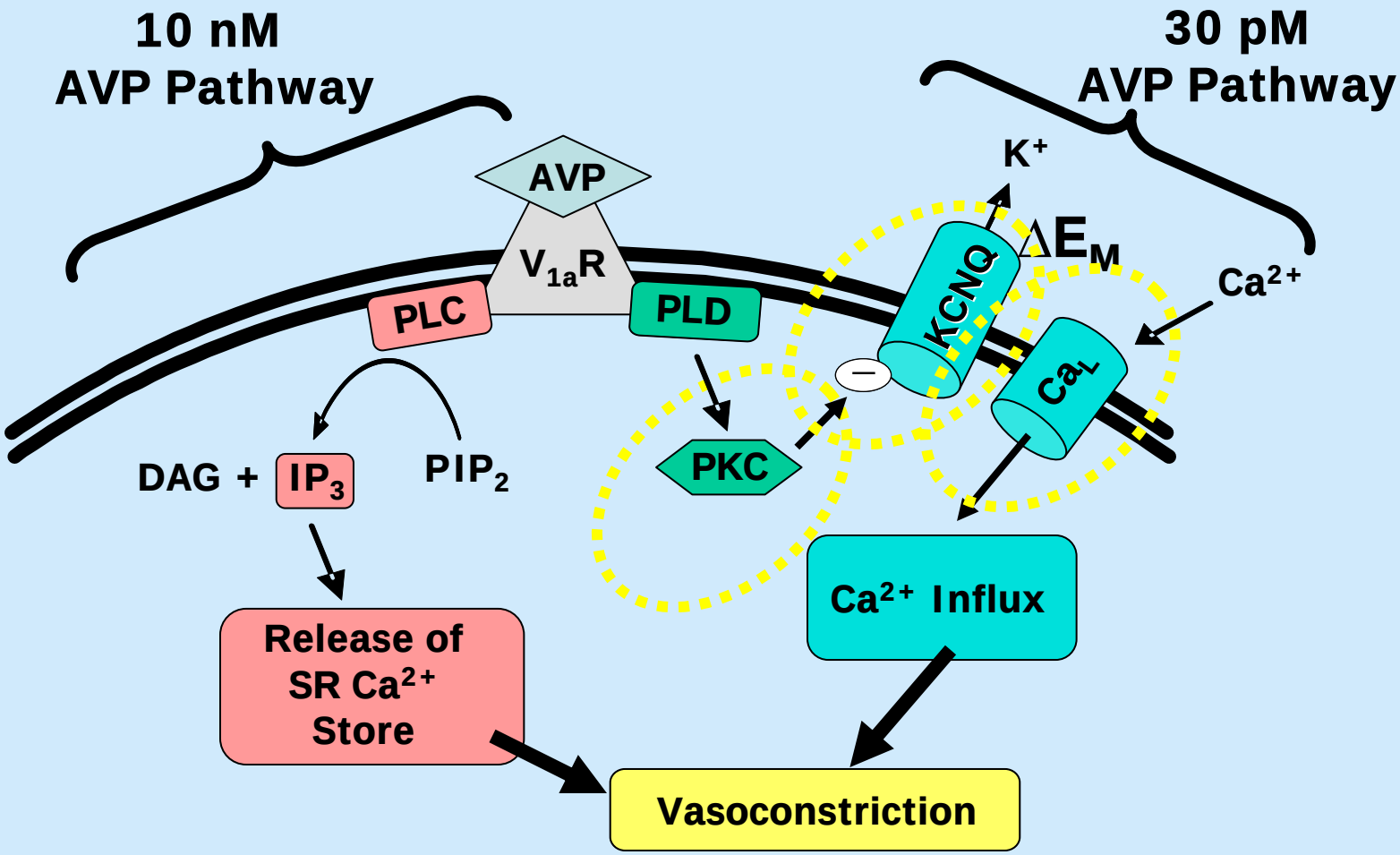




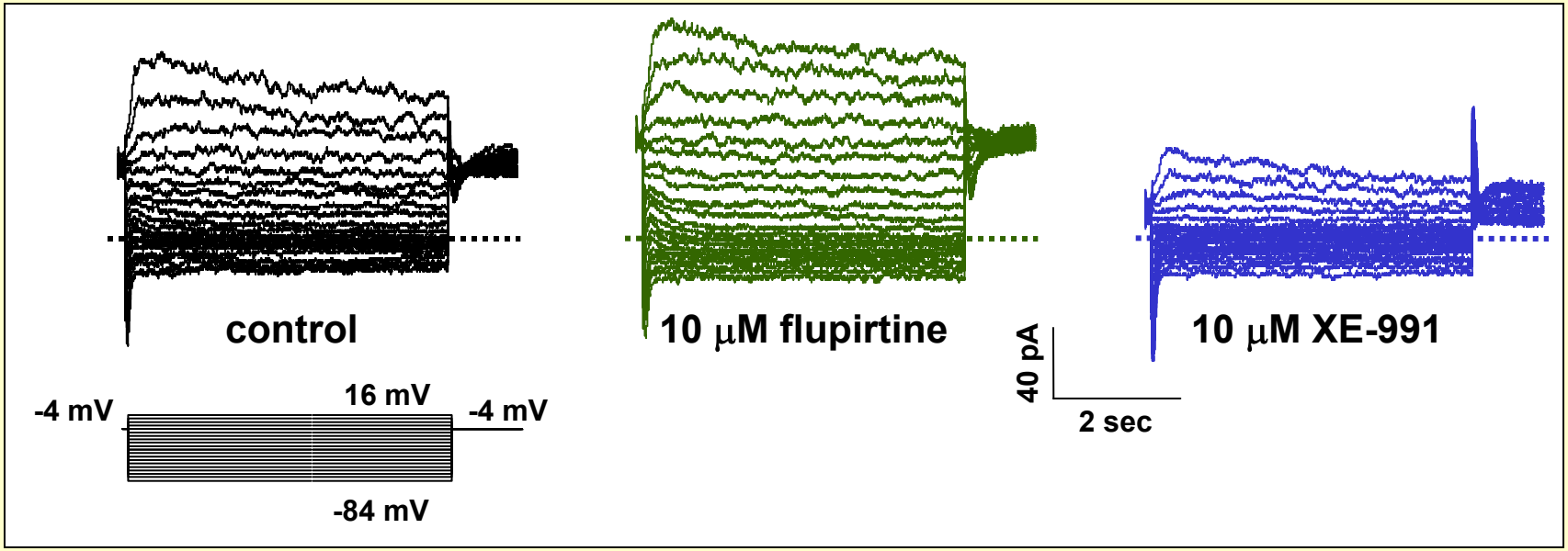


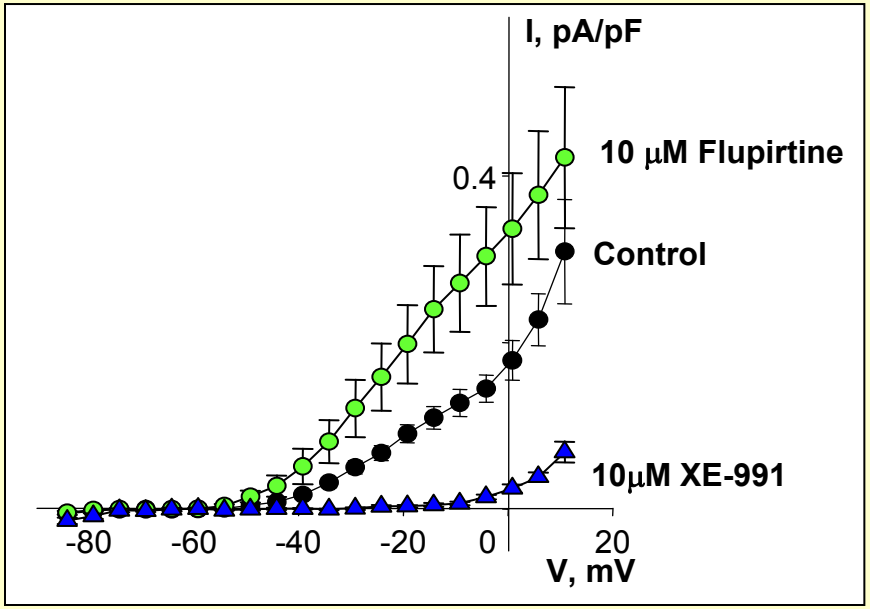
Henderson & Byron, J Appl Physiol 102:1402-1409, 2007

Novel AVP signaling pathway



M-currents measured in freshly isolated mesenteric artery myocytes

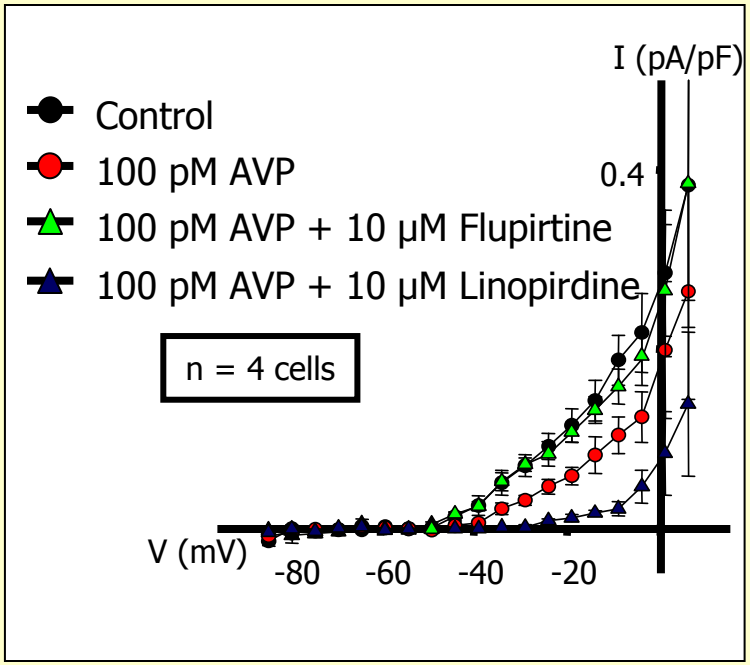


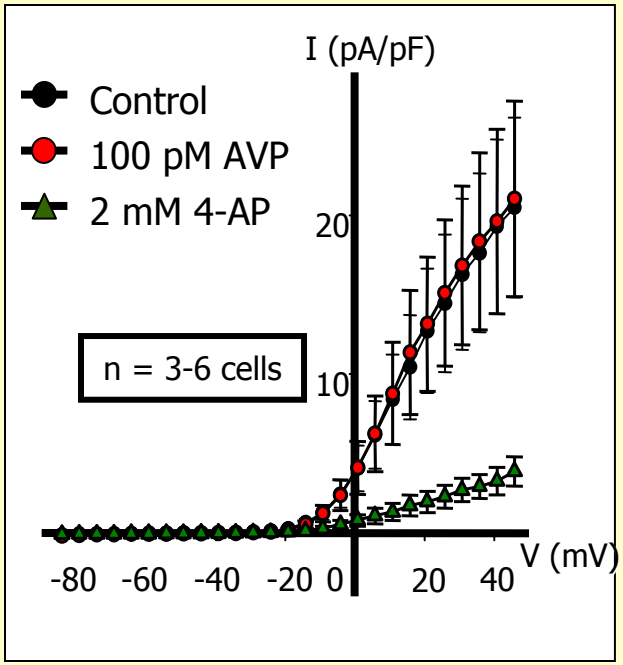


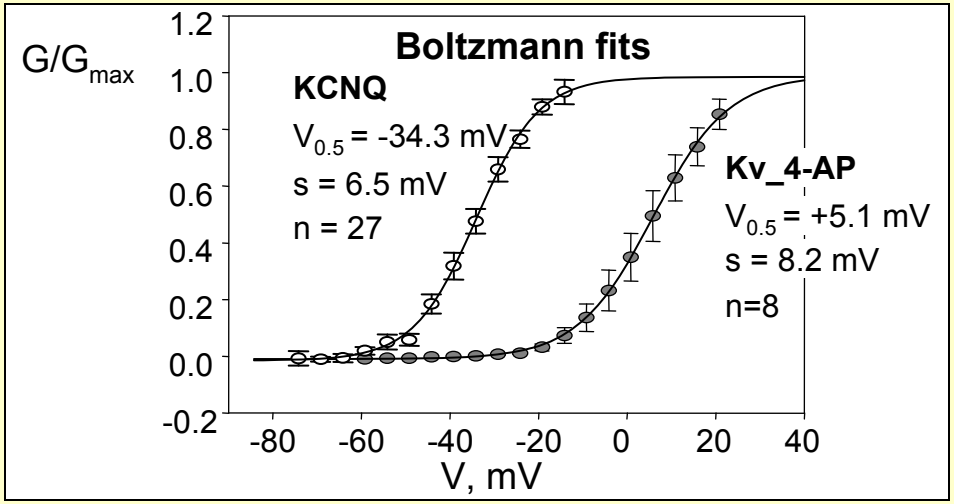
K_v currents are enhanced by KCNQ channel activators (**retigabine** and **flupirtine**) and abolished by KCNQ blockers (**XE991** and **linopirdine**).

Mackie *et al.*, *JPET* 2008

AVP suppresses KCNQ currents, but <u>not</u> 4-AP-sensitive K_v currents

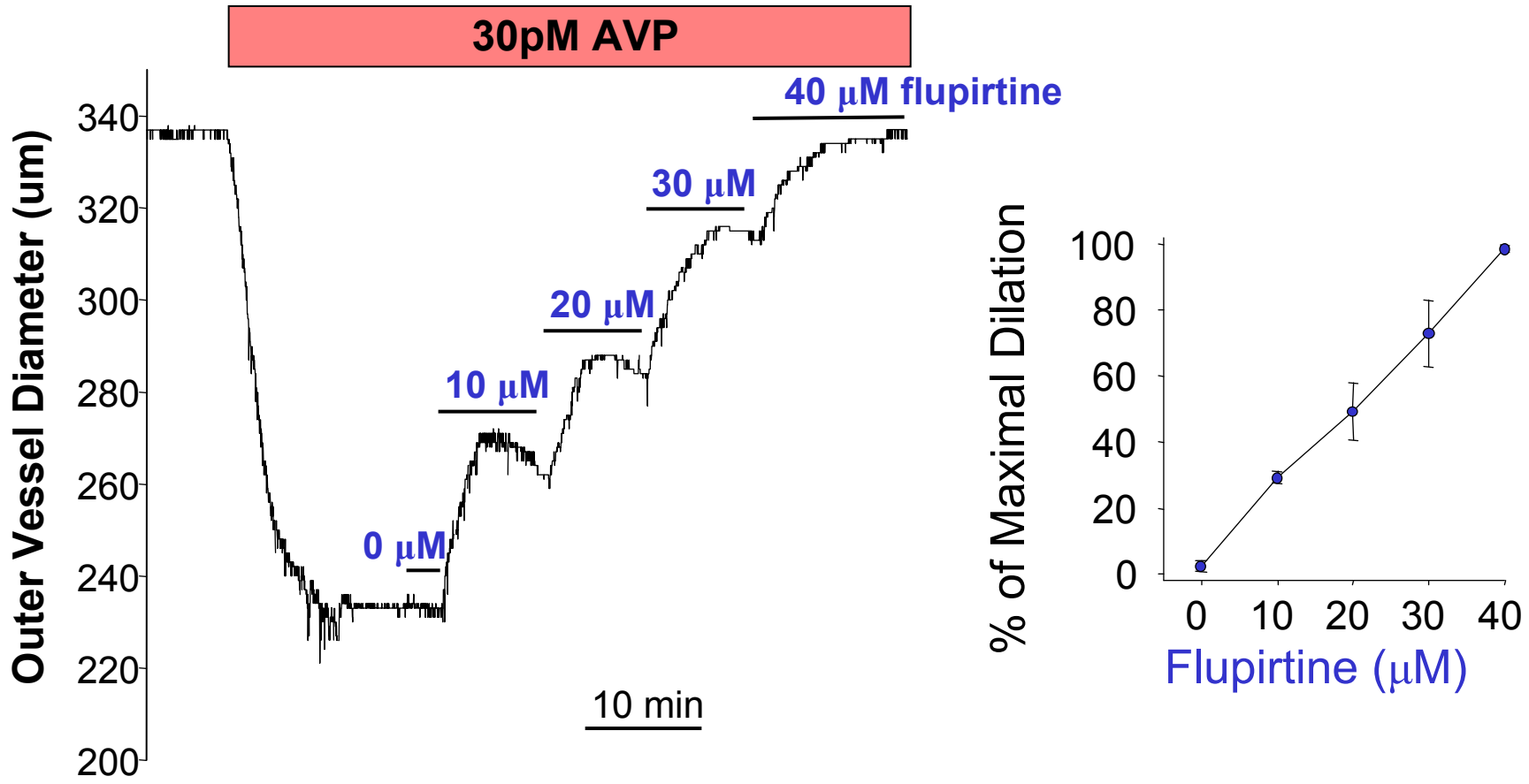






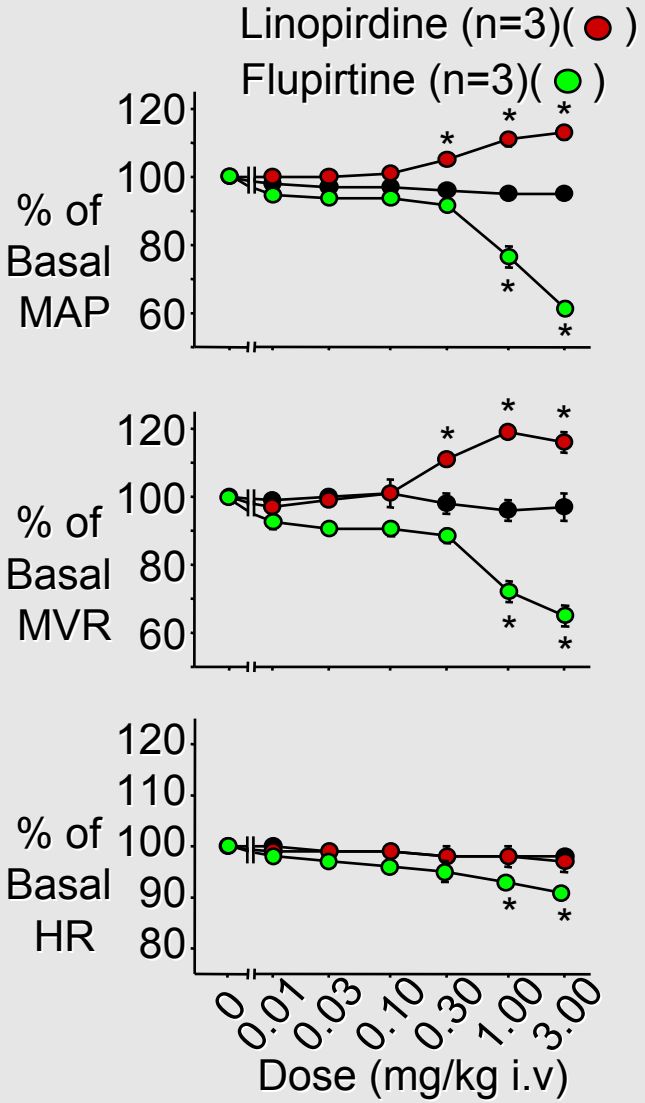
Mackie *et al.*, *JPET* 2008

KCNQ channel activator flupirtine: concentration-dependent vasodilation



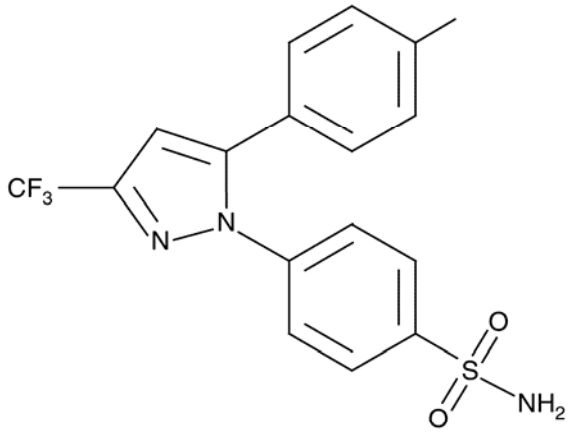
Mackie et al. *JPET* 2008

In Vivo Effects of KCNQ Channel Modulators

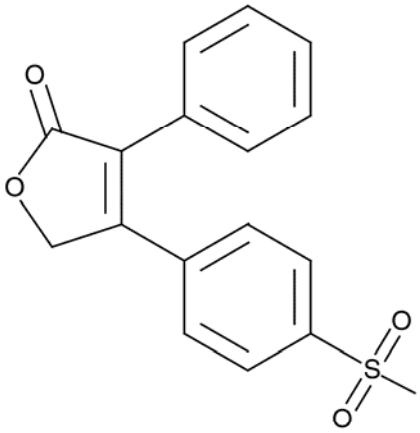


Mackie et al. *JPET* 2008

COX-2 Inhibitors

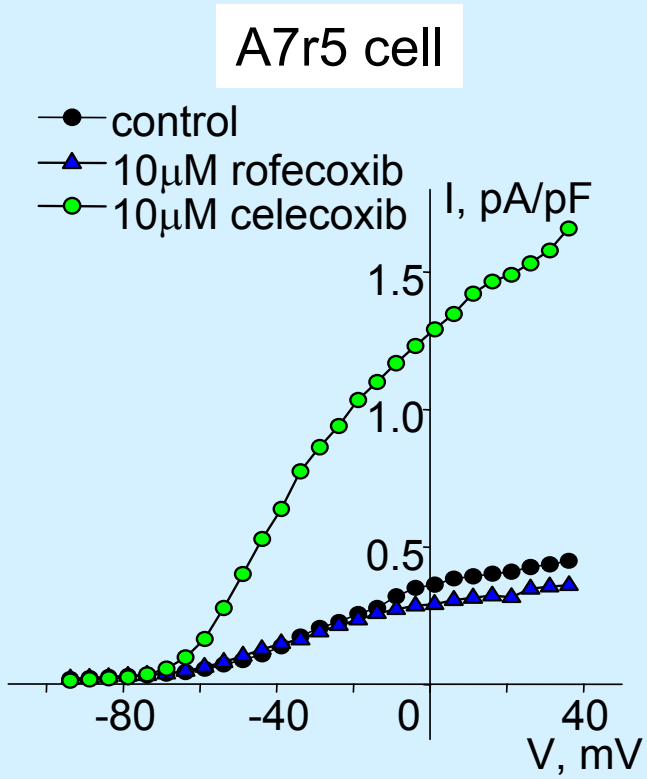


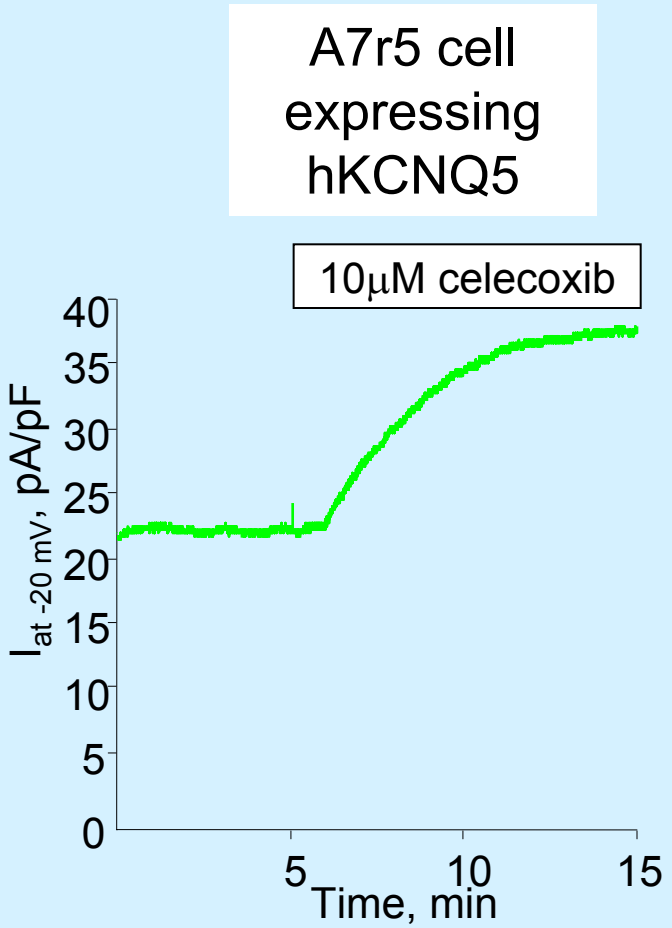
Celecoxib
(Celebrex®)



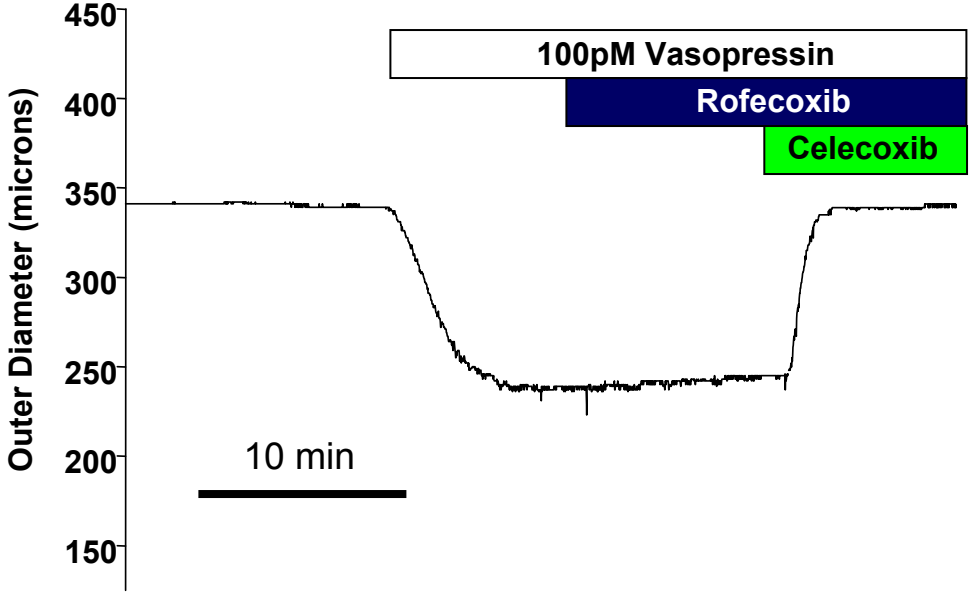
Rofecoxib
(Vioxx®)

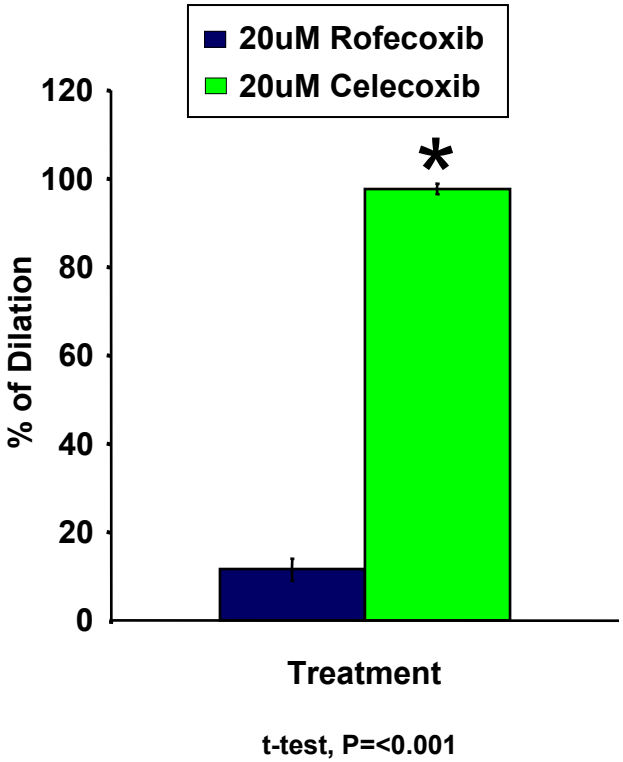
Celebrex®, but not Vioxx®, activates native or overexpressed vascular KCNQ channels





Celebrex® but not Vioxx® can relax rat mesenteric arteries pre-constricted with AVP

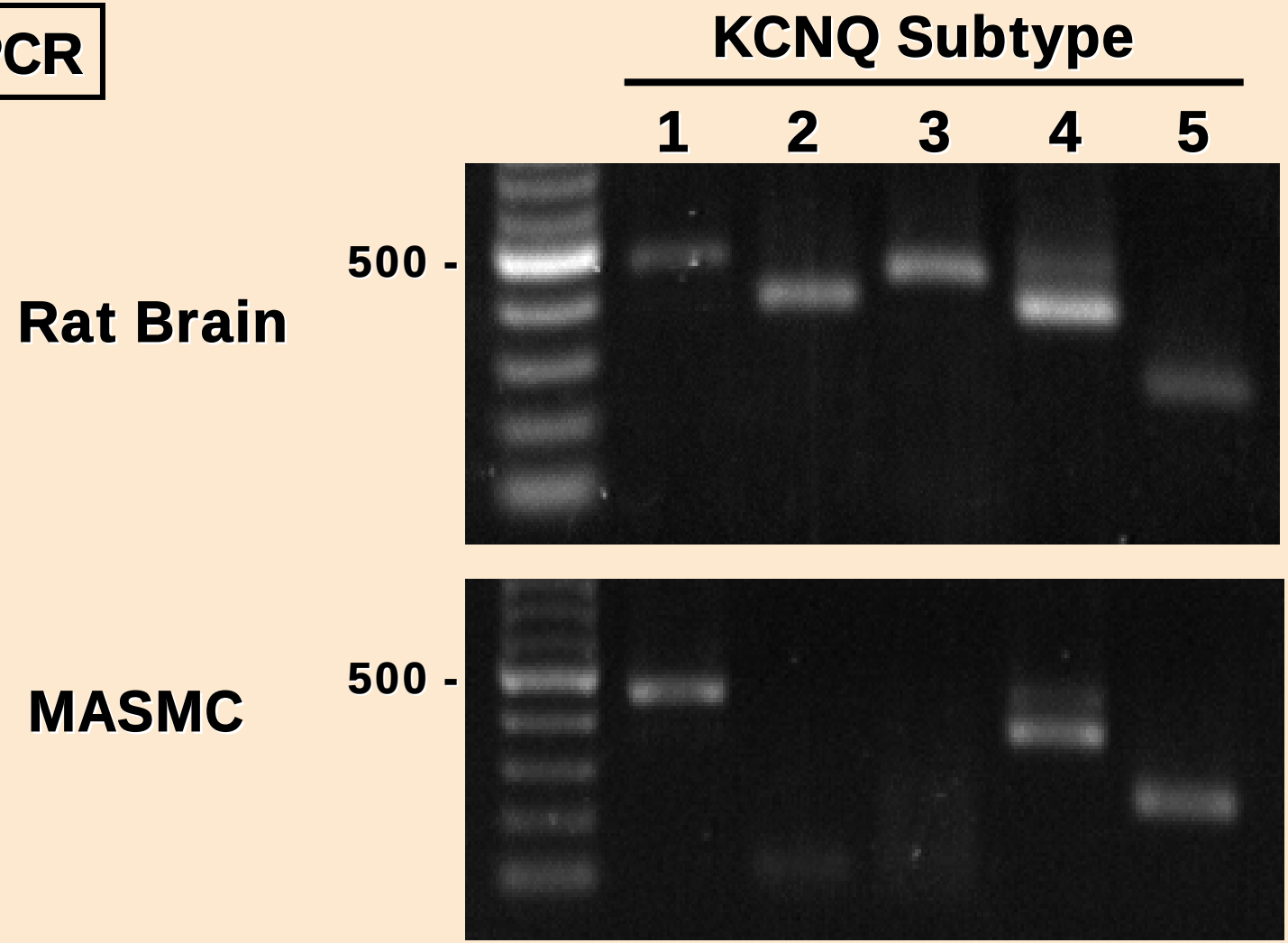




Brueggemann et al. *Mol Pharm* 2009

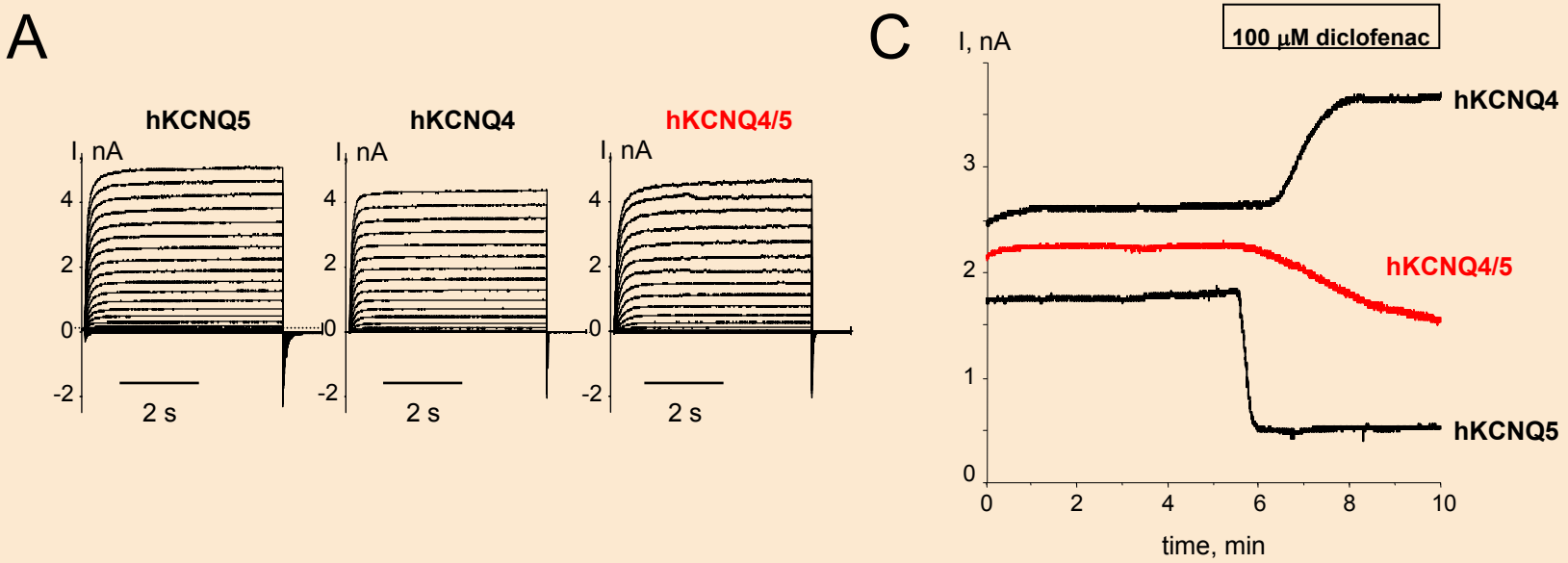
KCNQ channel expression in mesenteric artery myocytes: KCNQ1, KCNQ4, & KCNQ5 are detected by RT-PCR

RT-PCR



Mackie *et al.*, *JPET* 2008

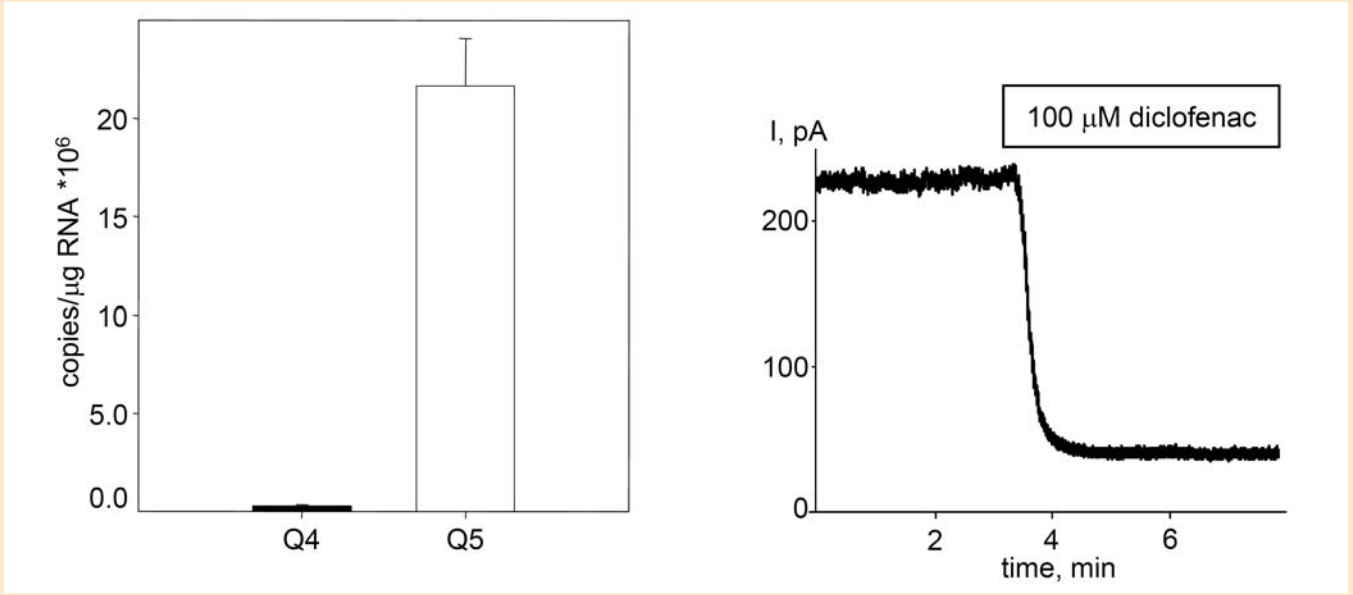
Diclofenac distinguishes between overexpressed homomeric and heteromeric KCNQ4 & KCNQ5 channels



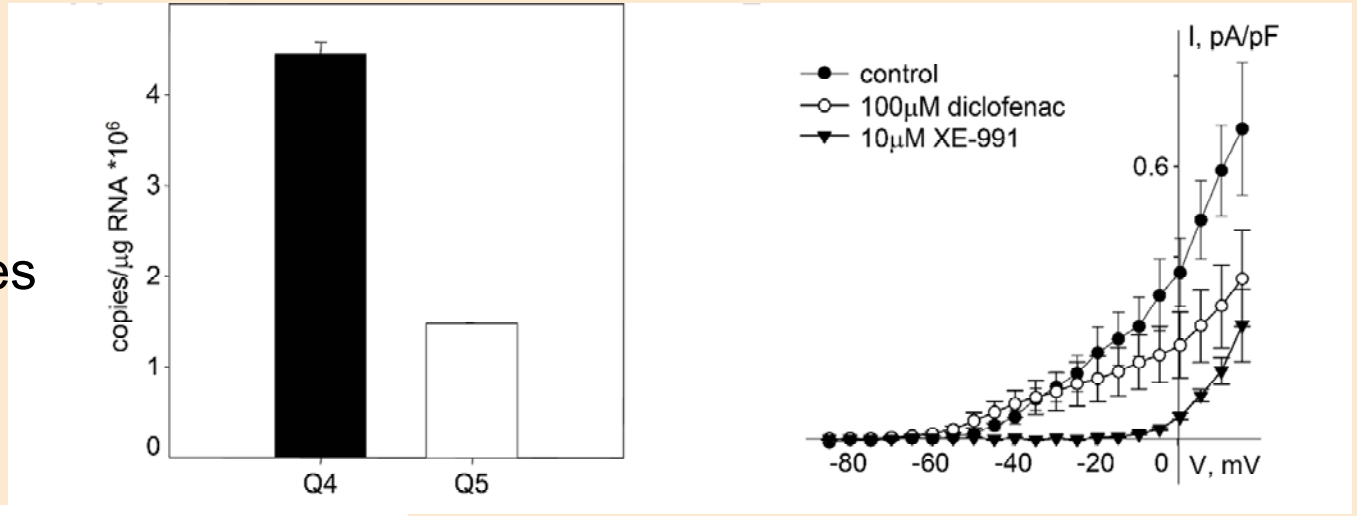
Brueggemann *et al.* Mol. Pharmacol., 79: 10-23, 2011.

Diclofenac distinguishes between natively expressed homomeric and heteromeric KCNQ4 & KCNQ5 channels

A7r5 cells

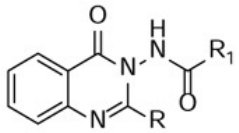
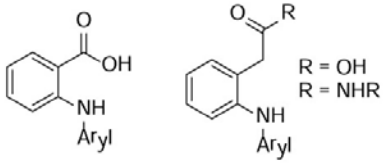


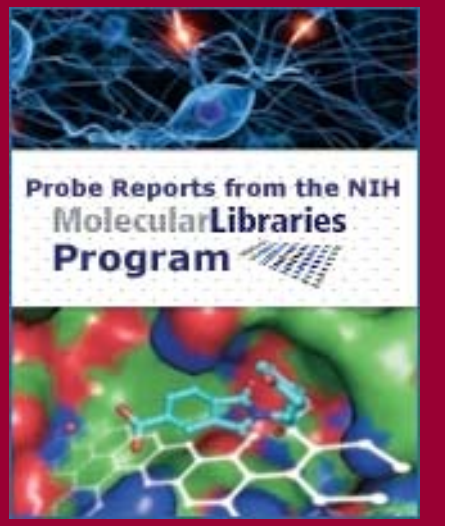
Mesenteric Artery myocytes



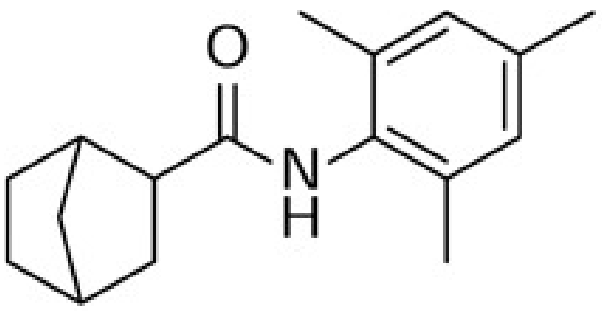
Brueggemann *et al.* Mol. Pharmacol., 79: 10-23, 2011.

LIST OF US PATENTS ISSUED PRIOR TO 2011 THAT CLAIM COMPOUNDS ACTIVATING KCNQ CHANNELS

Patent number	Company	Target channel	Chemical class	Representative compound
6,326,385	Icagen	KCNQ2/Q3	N-aryl benzamide	ICA-27243 analogs
6,372,767	Icagen	KCNQ2	Benzanilides and 2-substituted-5-aminopyridines	
6,495,550	Icagen	KCNQ2	Pyridine-substituted benzanilides	
6,989,398	Icagen	KCNQ2	Benzanilides	
6,605,725	Icagen	KCNQ2	Benzanilides	
6,737,422	Icagen	KCNQ2	Benzanilides	
6,593,349	Icagen	KCNQ2	Bisarylamines	
7,741,332	Icagen	KCNQ2	Fused ring heterocycles	
7,223,768	Icagen	KCNQ2	Fused ring heterocycles	
6,469,042	BMS	KCNQ2, KCNQ2/Q3	Fluoro oxindole derivatives	BMS-204352 analogs
6,855,829	BMS	KCNQ2, KCNQ5	3-fluoro-2-oxindole and 2,4-disubstituted pyrimidine-5-carboxamide	
7,632,866	Tel Aviv U.	KCNQ2/3, Q1, Q1/E1	Derivatives of N-phenylanthranilic acid and 2-benzimidazolone as **potassium** channel and/or neuron activity modulators	



ML213



N-mesitylbicyclo[2.2.1]heptane-2-carboxamide

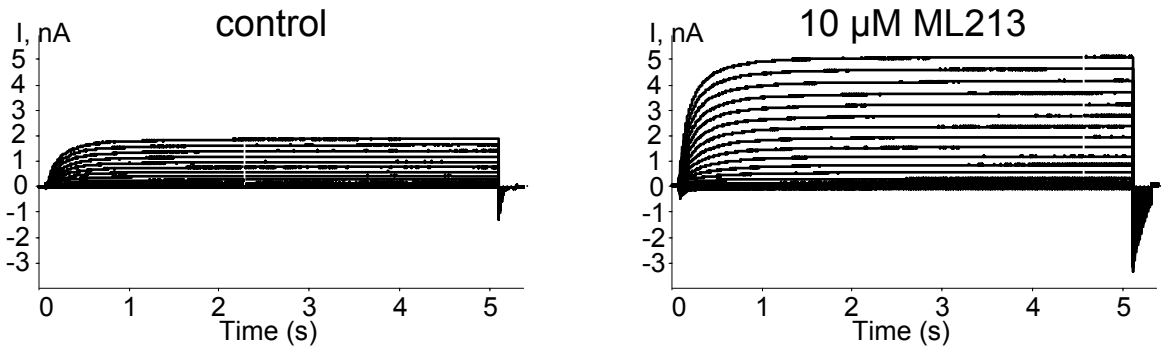
A small molecule activator of KCNQ2 and KCNQ4 channels

Haibo Yu, Meng Wu, Corey Hopkins, Julie Engers, Steve Townsend, Craig Lindsley, Owen B McManus, and Min Li.

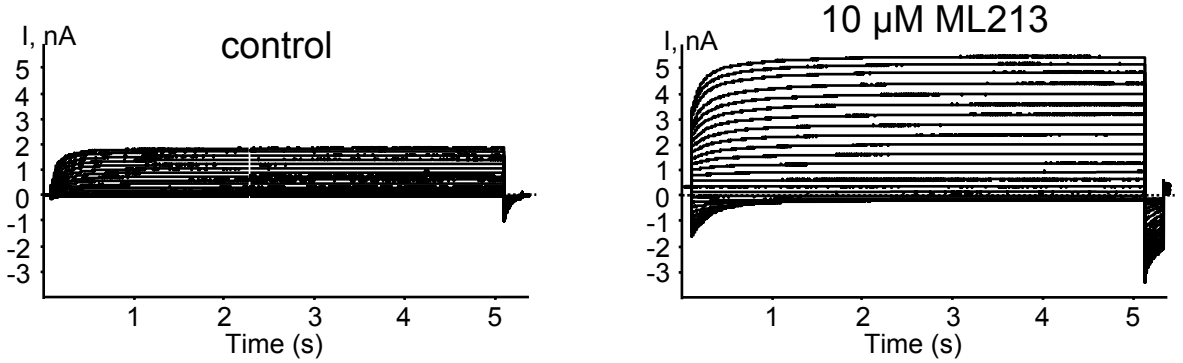
"ML213 was identified following a high throughput fluorescent screen of the Molecular Libraries Small Molecule Repository (MLSMR) library and structure activity relationship (SAR) studies using fluorescent and electrophysiological assays to determine potency and selectivity of test compounds. ML213 is a potent activator of potassium voltage-gated channel, KQT-like subfamily, member 2 (KCNQ2) (Kv7.2, EC50 = 230 nM) and KCNQ4 (Kv7.4, EC50 = 510 nM) and selective against the other members of the KCNQ family of ion channels (KCNQ1, KCNQ3 and KCNQ5)."

Probe Reports from the NIH Molecular Libraries Program

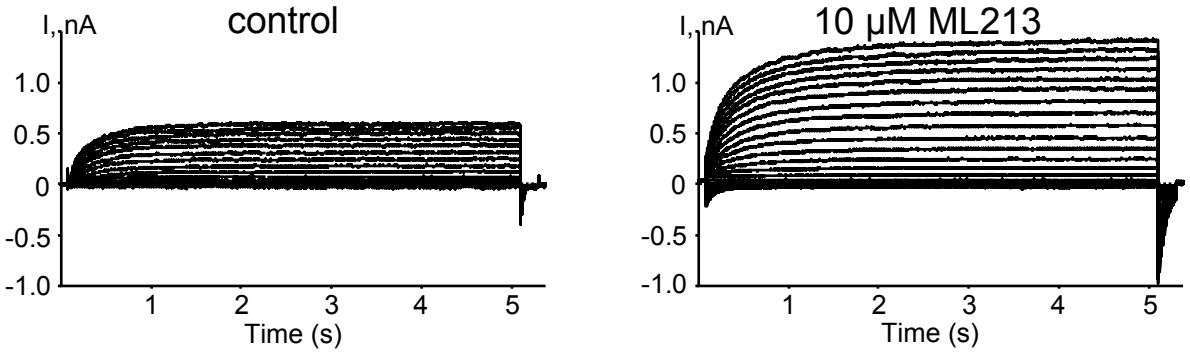
hKv7.4



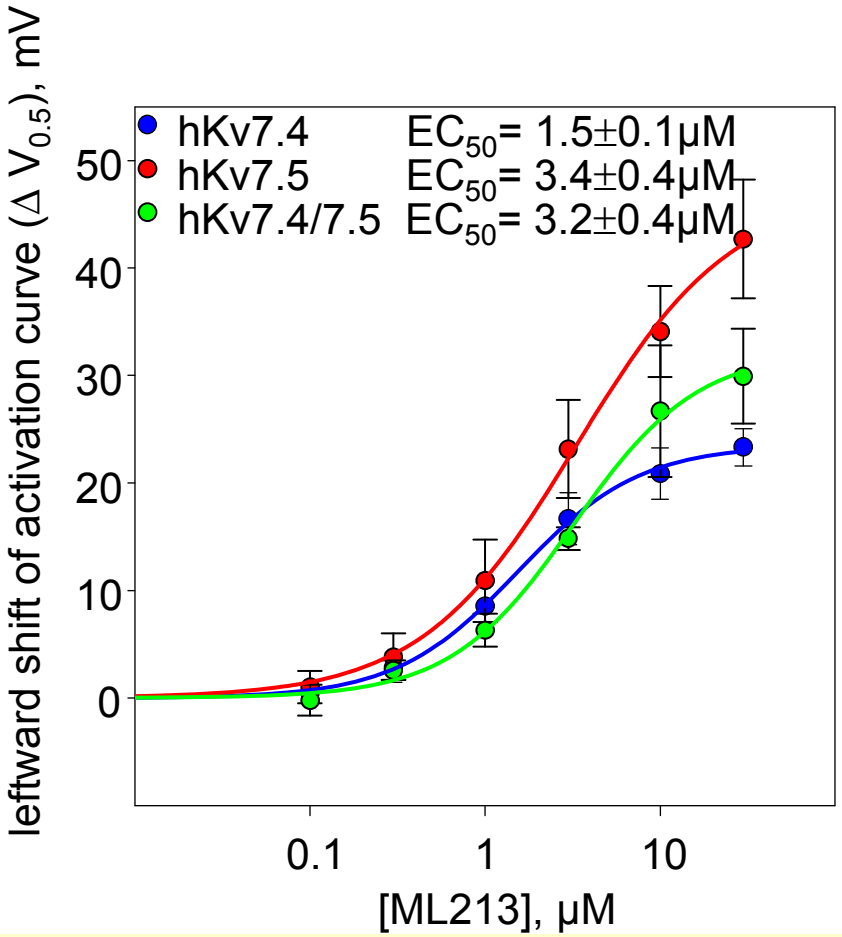
hKv7.5



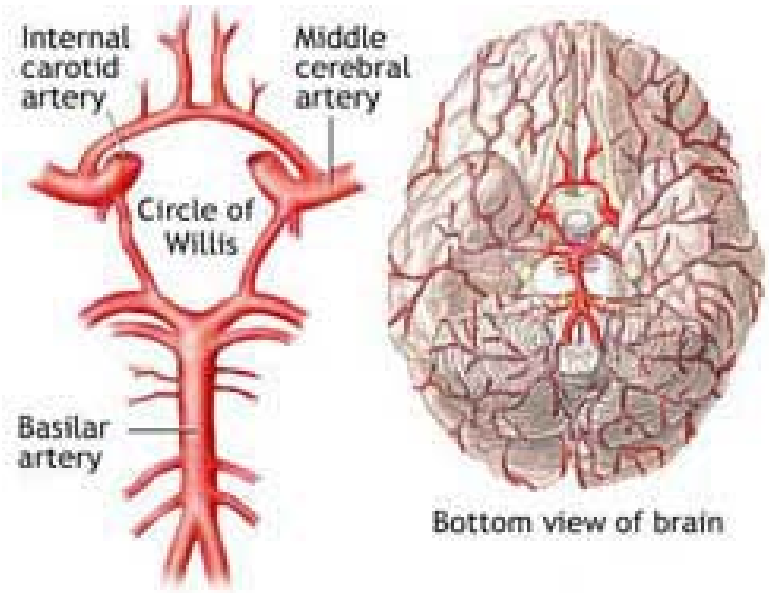
hKv7.4/7.5

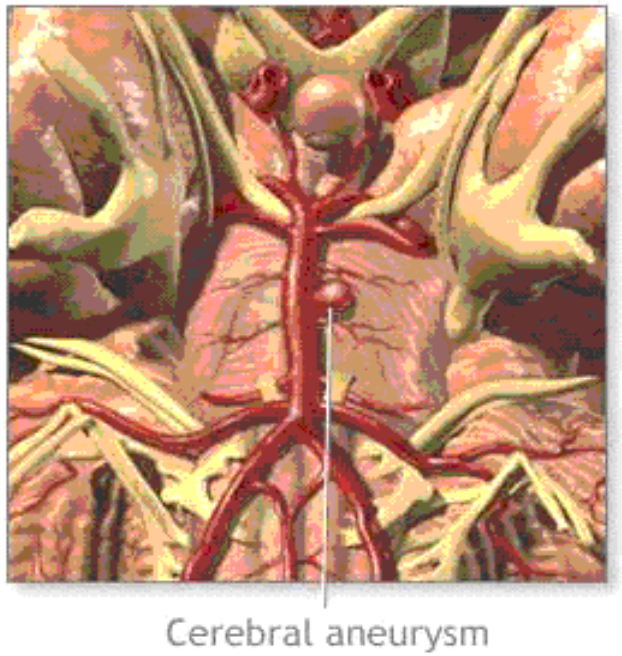


ML213

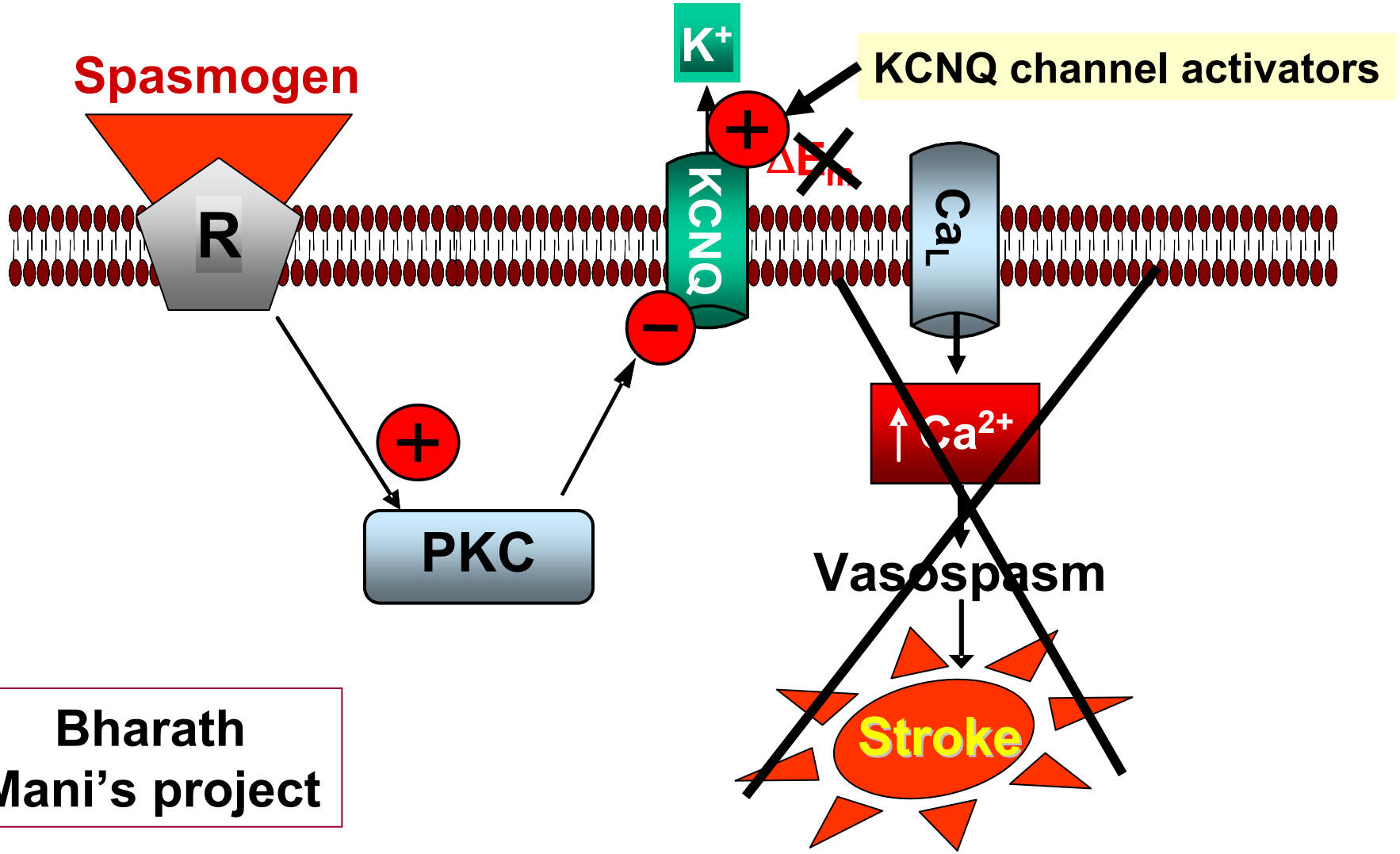

hKv7.4 EC50= 1.5±0.1μM
hKv7.5 EC50= 3.4±0.4μM
hKv7.4/7.5 EC50= 3.2±0.4μM
leftward shift of activation curve (Δ V0.5), mV
50
40
30
20
10
0
0.1
1
10
[ML213], μM

Cerebral Aneurysm and Subarachnoid hemorrhage (SAH).



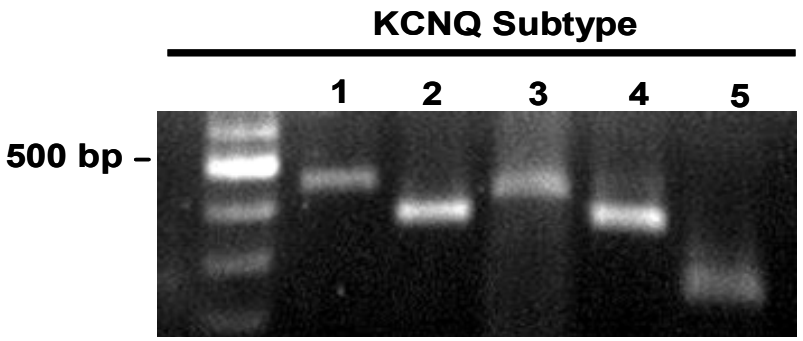


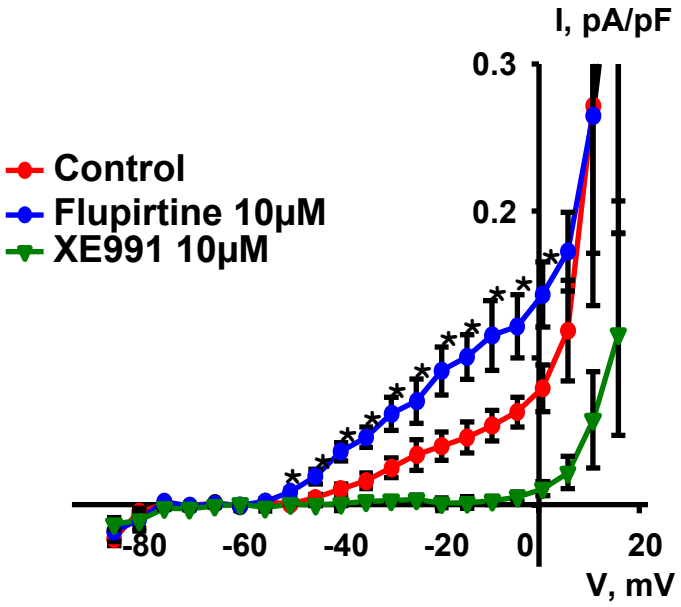
Hypothetical model of the role of KCNQ channels in cerebral vasospasm.



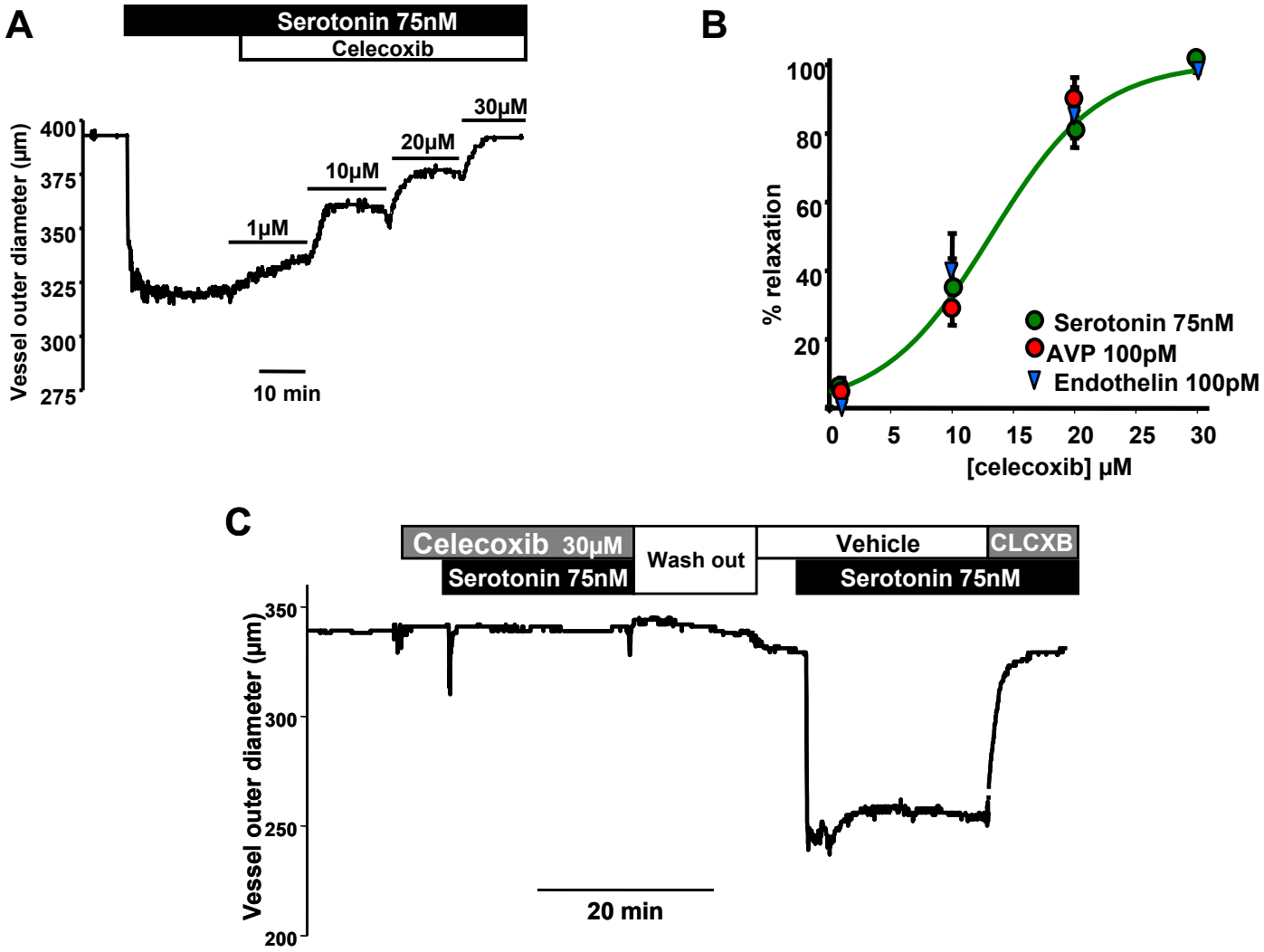
Bharath
Mani's project

KCNQ channels are expressed and functional in basilar artery myocytes.

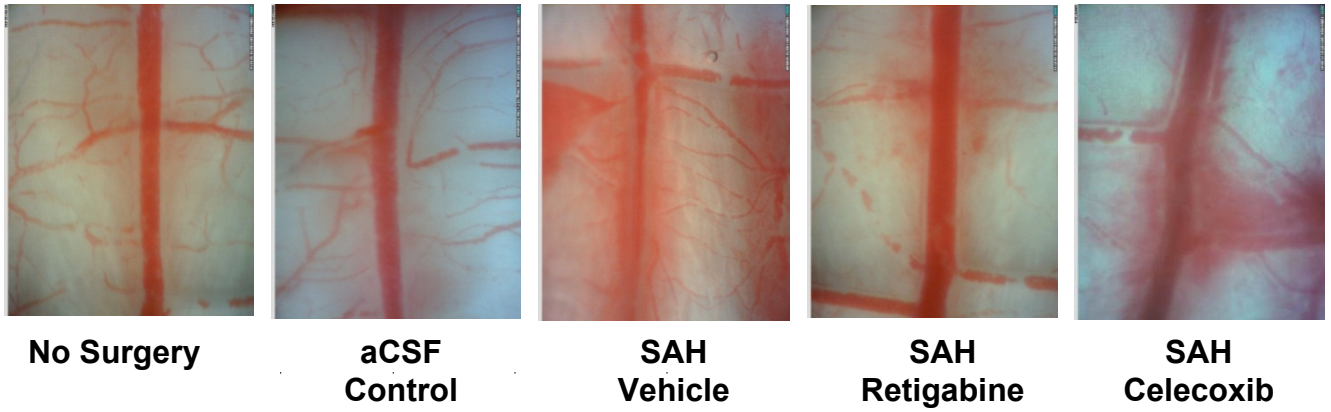


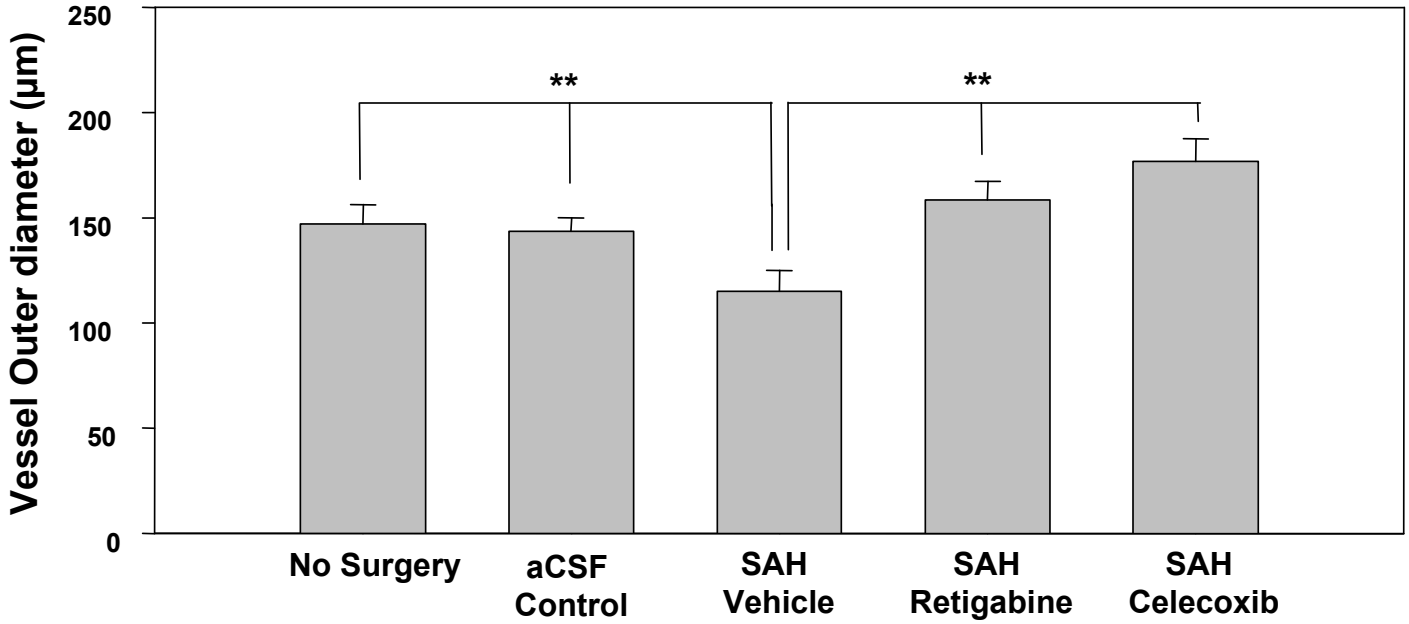


Celebrex® prevents or reverses vasoconstriction in basilar arteries



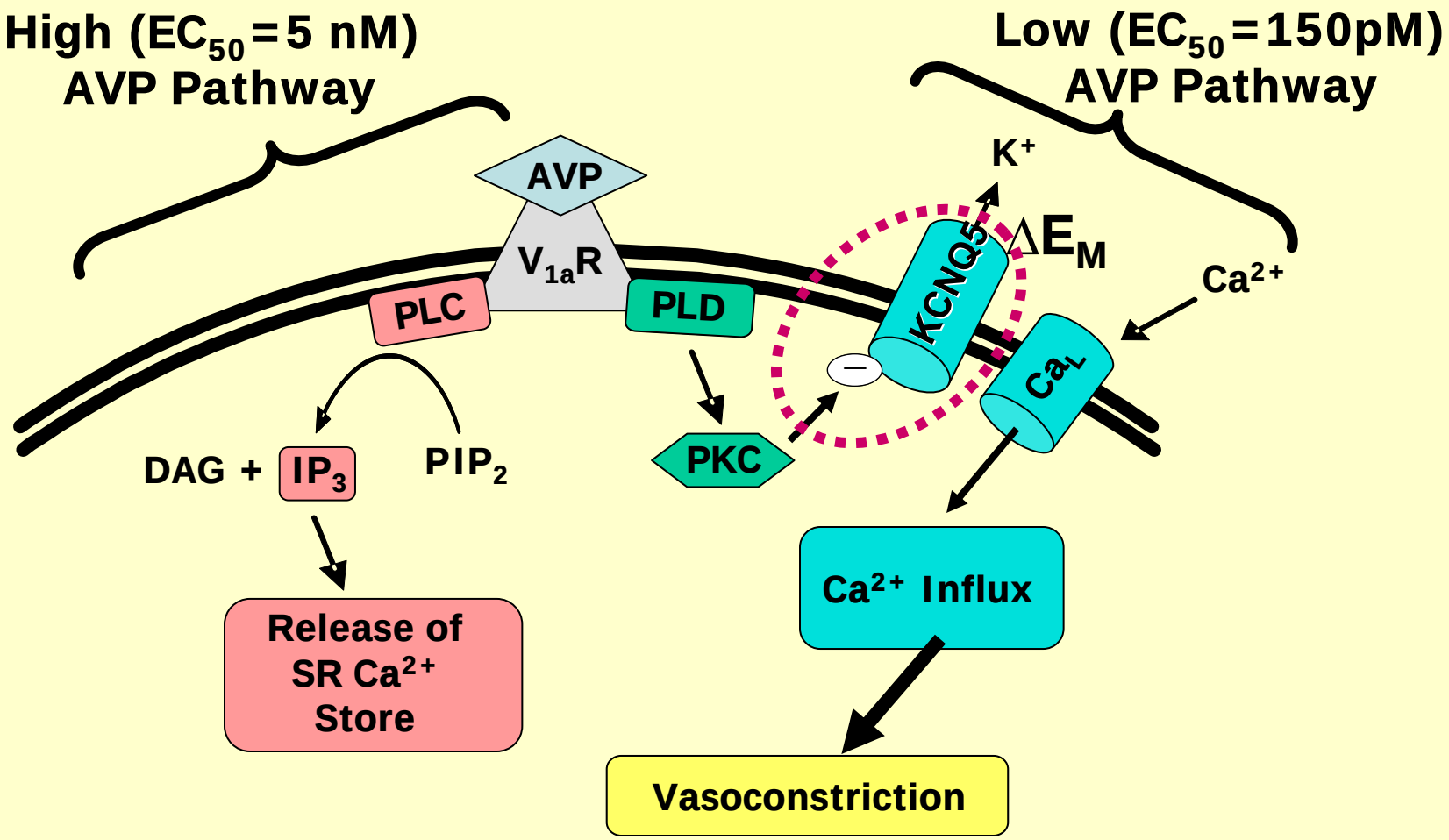
Retigabine or Celecoxib prevents basilar artery vasospasm in a rat model of SAH



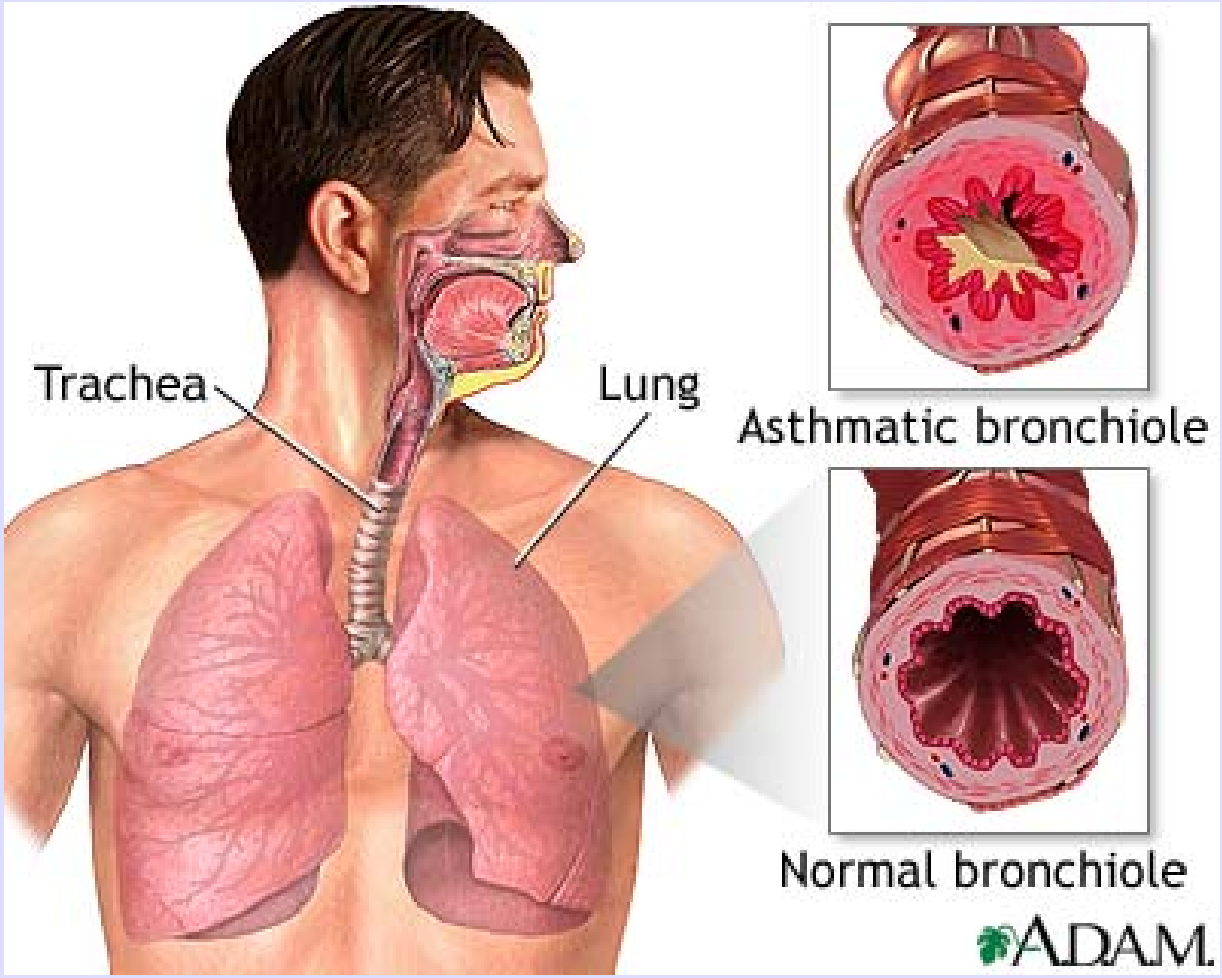


Mani *et* al. (2013) J. *Cardiovasc. Pharmacol.* **61**, 51-62

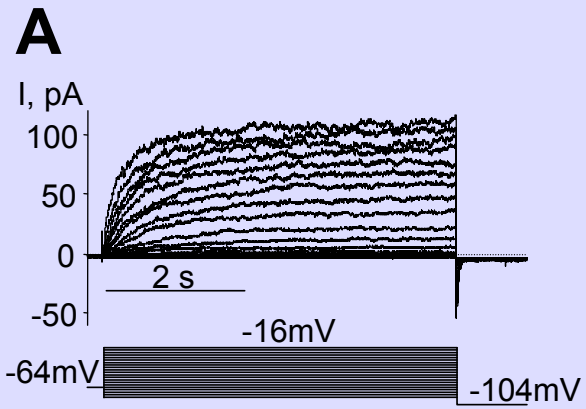
KCNQ CHANNELS IN VASCULAR SMOOTH MUSCLE CELLS AS PHYSIOLOGICAL AND THERAPEUTIC TARGETS

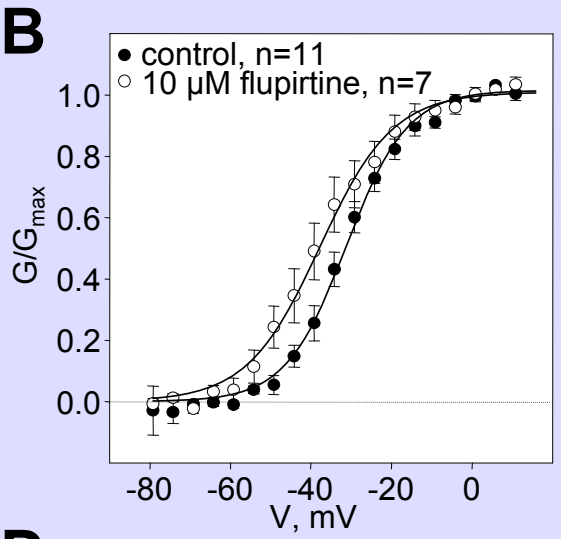


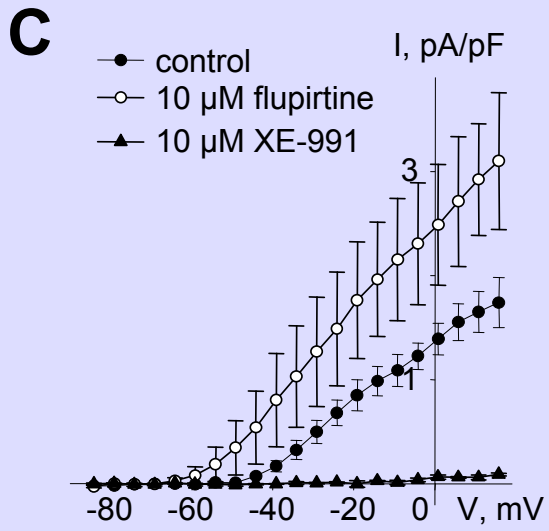
KCNQ CHANNELS IN *AIRWAY* SMOOTH MUSCLE CELLS AS PHYSIOLOGICAL AND THERAPEUTIC TARGETS?

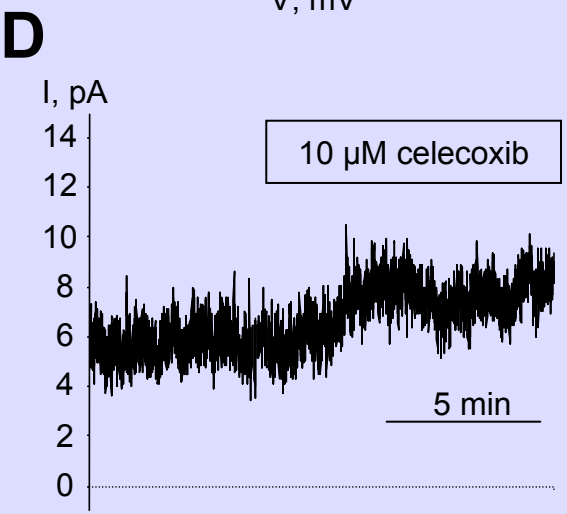


Kv7 CURRENTS IN AIRWAY SMOOTH MUSCLE CELLS ARE ENHANCED BY FLUPIRTINE AND CELECOXIB.

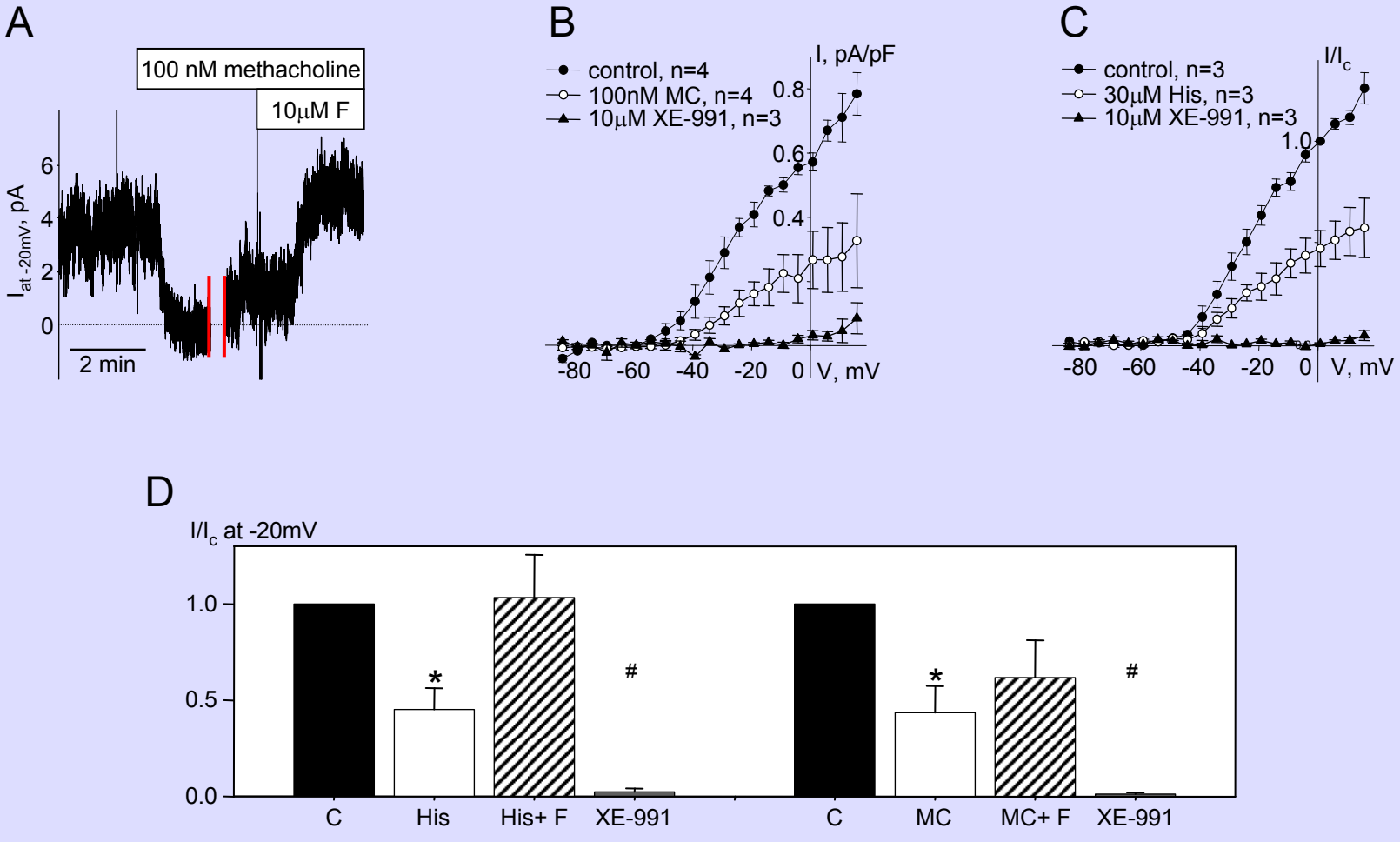






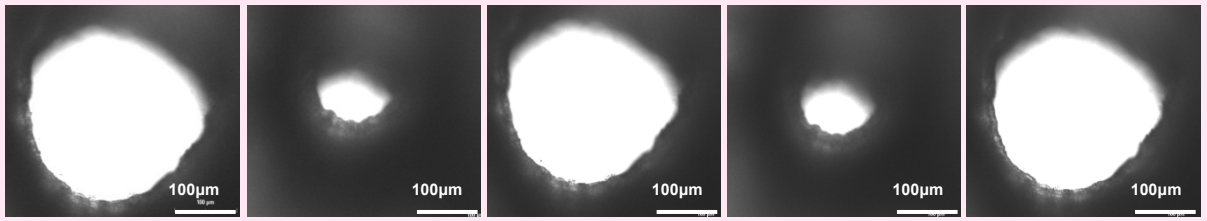


SUPPRESSION OF Kv7 CURRENTS IN AIRWAY SMOOTH MUSCLE CELLS BY METHACHOLINE AND HISTAMINE IS REVERSED BY FLUPIRTINE.



PRECISION-CUT LUNG SLICES: FUNCTIONAL EFFECTS OF KCNQ CHANNEL MODULATION ON AIRWAY DIAMETER

A



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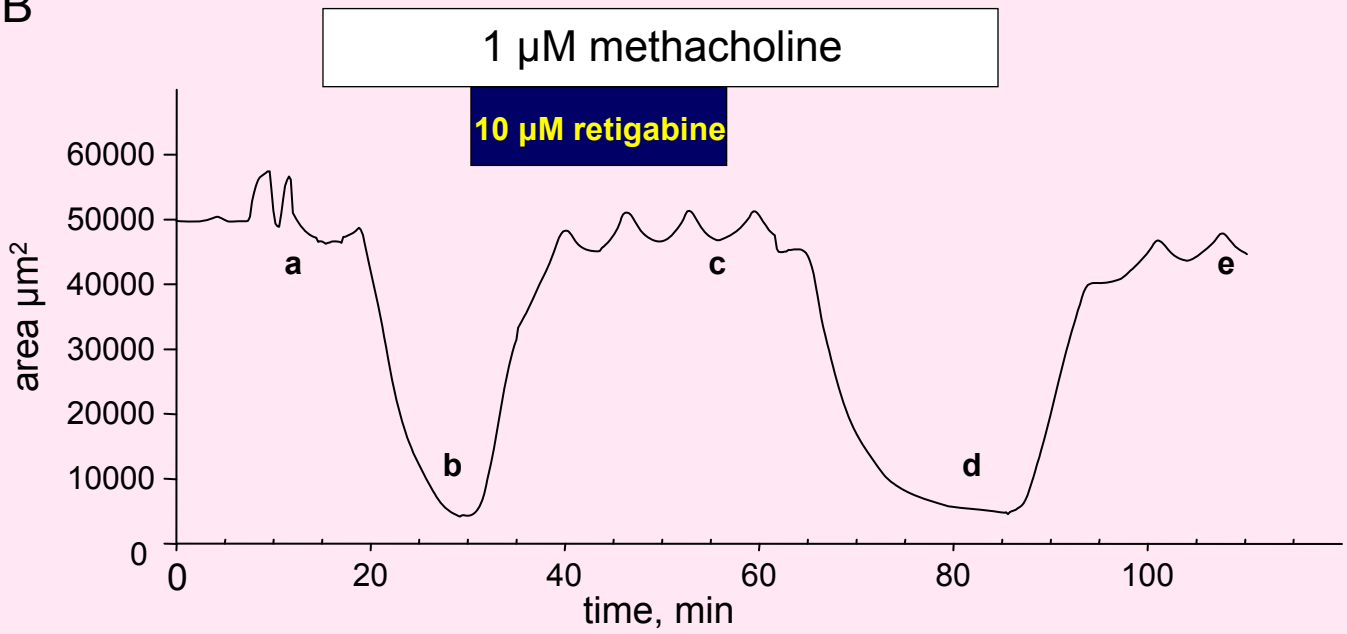
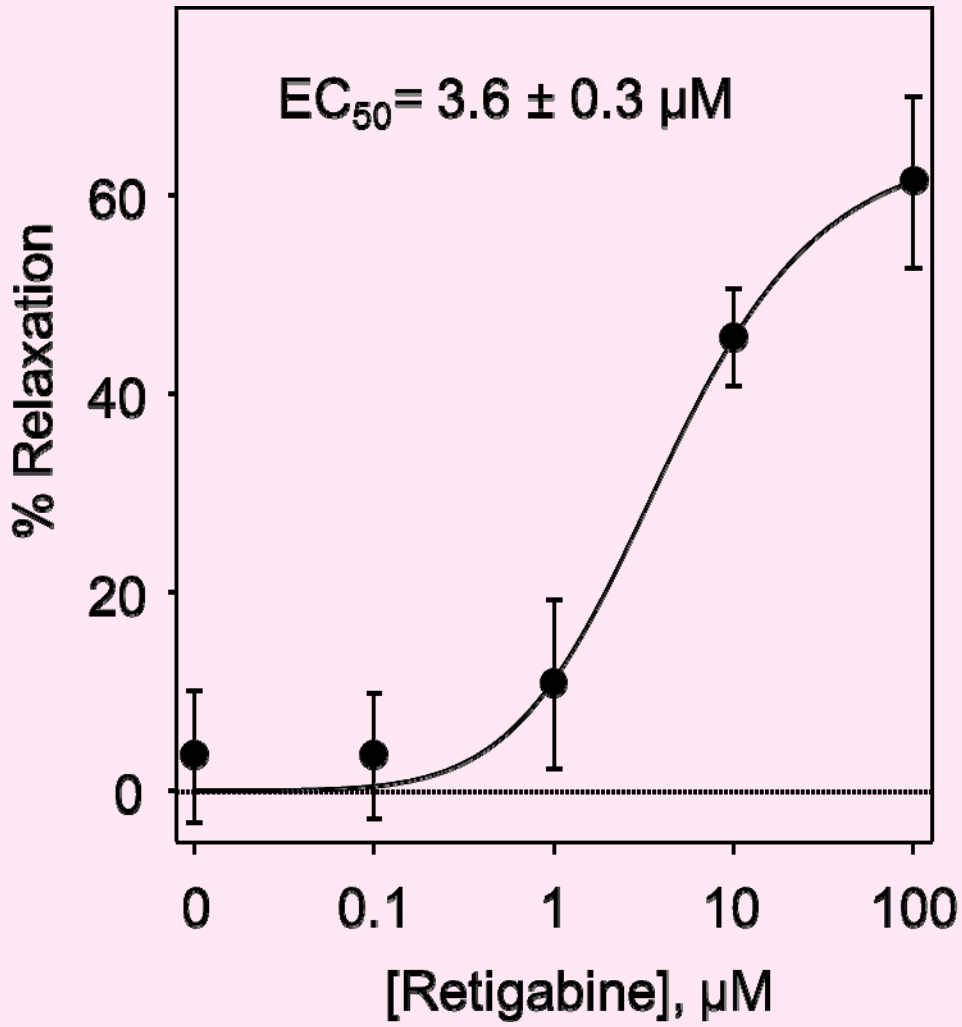


CONCENTRATION-DEPENDENCE OF REIGABINE-INDUCED RELAXATION OF RAT AIRWAYS



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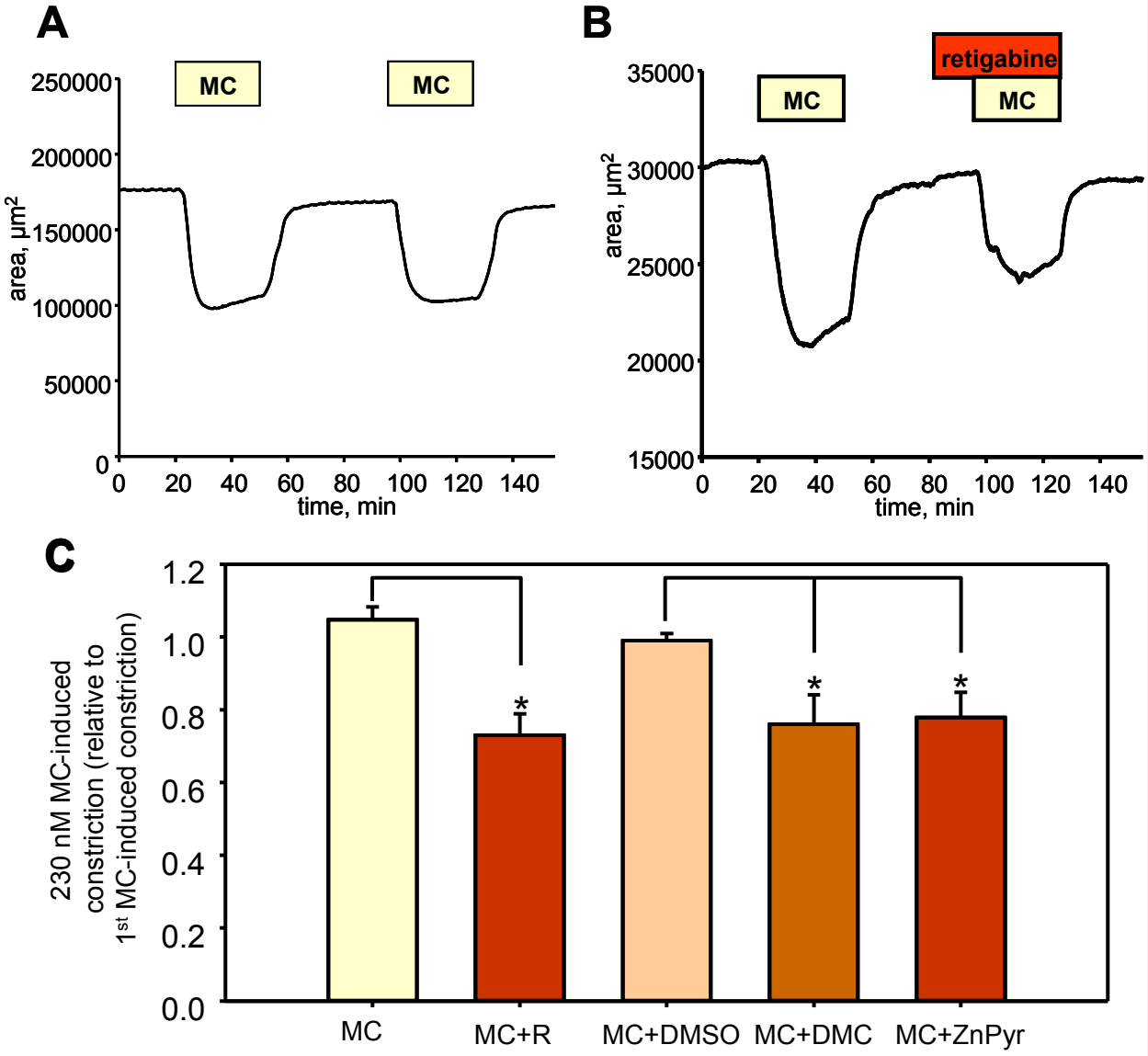
KCNQ POTASSIUM CHANNEL ACTIVATORS ATTENUATE METHACHOLINE-INDUCED CONSTRICTION OF RAT AIRWAYS.



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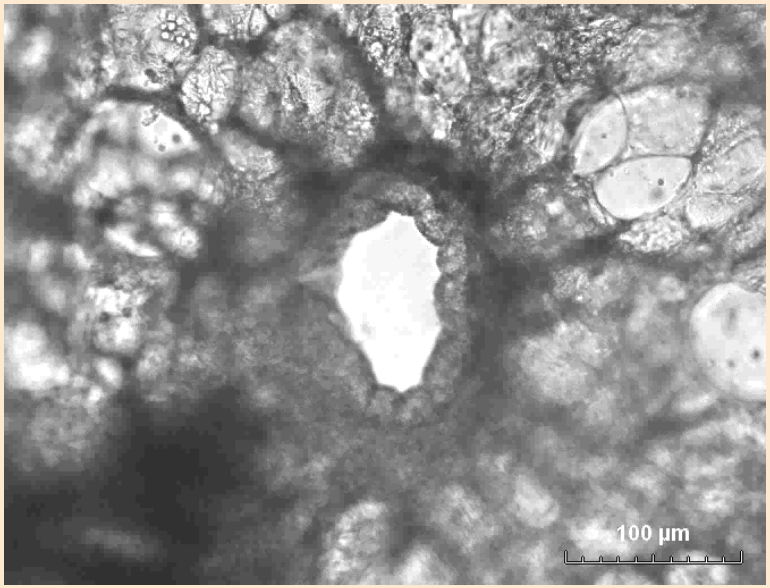
PROFOUND CONSTRICTION OF HUMAN AIRWAYS INDUCED BY Kv7 CHANNEL BLOCKER XE991; ATTENUATION OF HISTAMINE-INDUCED AIRWAY CONSTRICTION BY FLUPIRTINE.



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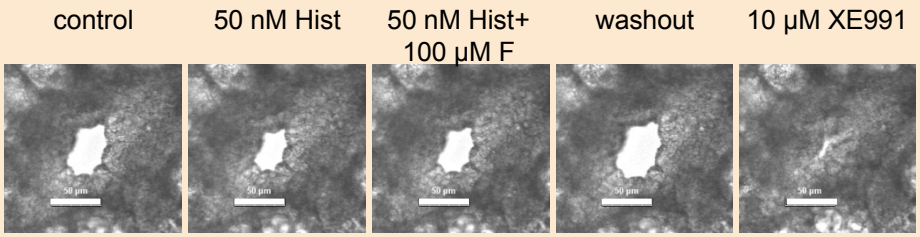

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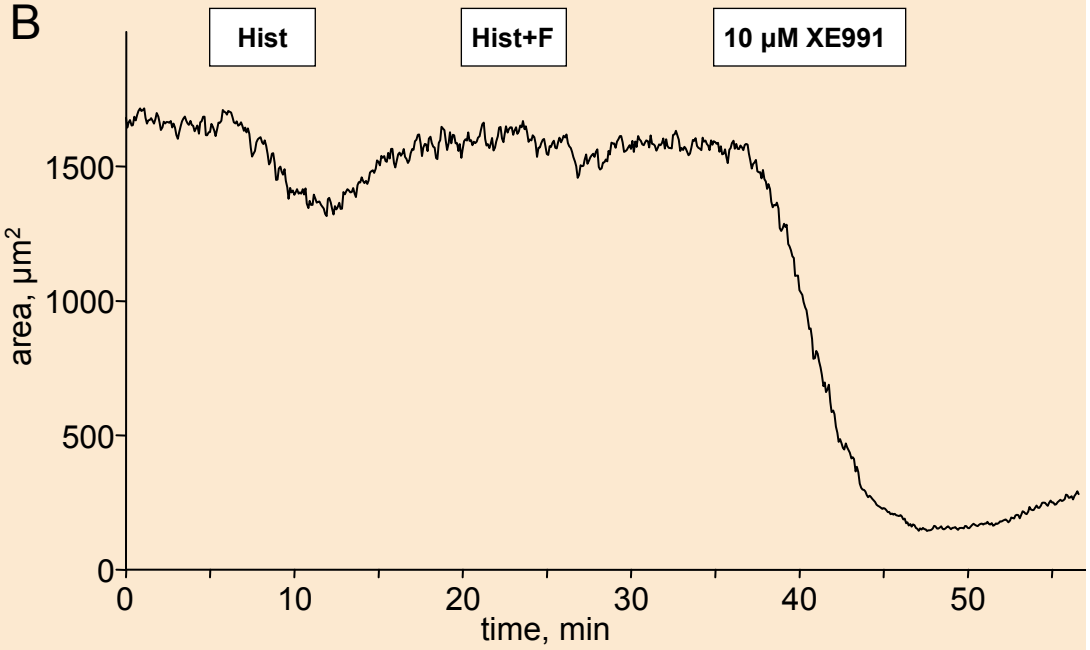


FORMOTEROL

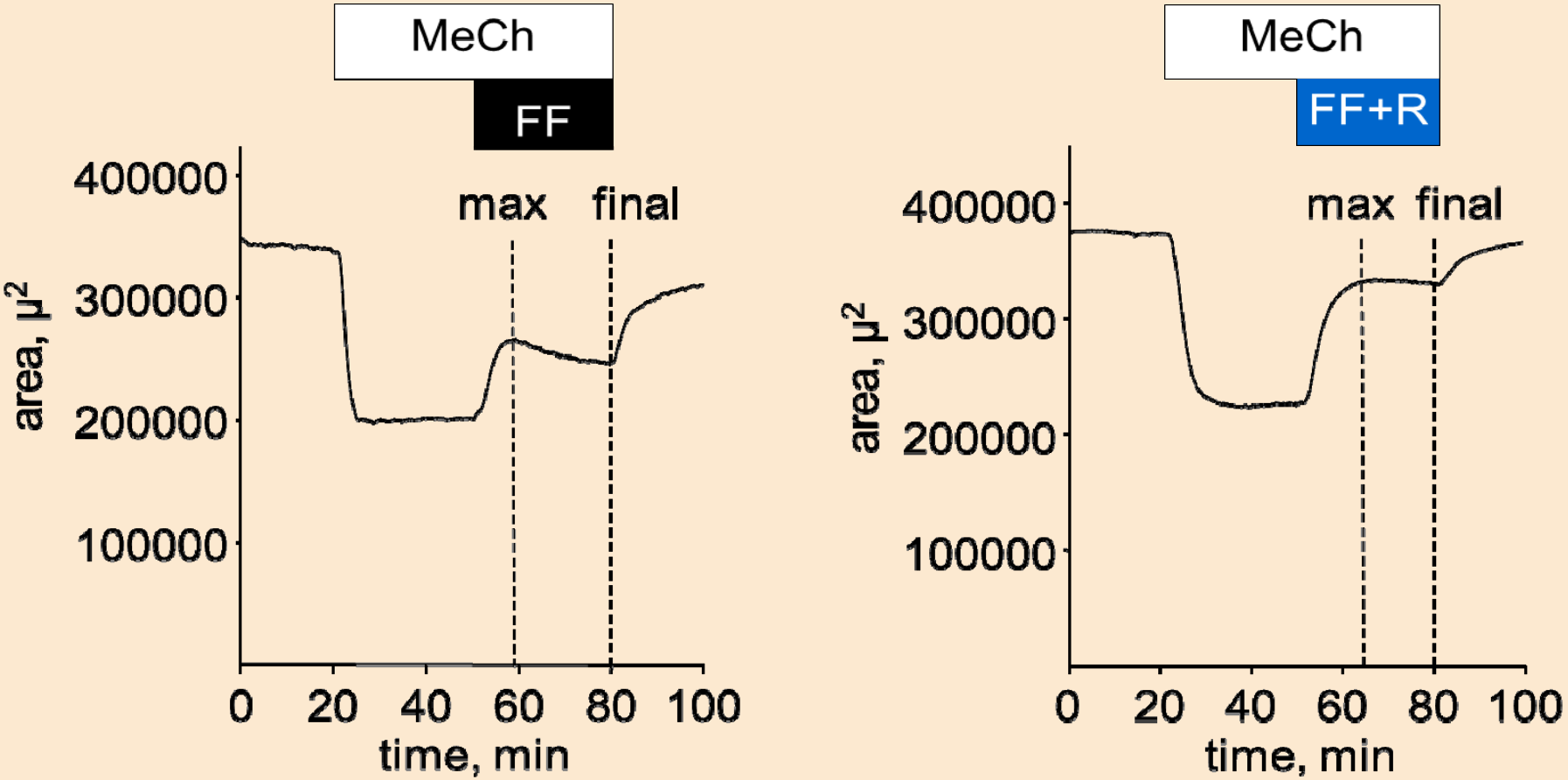
Drug class: Long-acting β_2-adrenergic agonist (LABA)

Use: Bronchospasm, Exercise-induced asthma

DESENSITIZATION TO LABAs

Regular treatment with both long- and short-acting β_2-agonists results in tolerance to their bronchoprotective effects. Twice daily administration of long-acting β_2-agonists results in blunted responses to repeated doses of short-acting β_2-agonists , as with rescue inhalers used in the setting of an acute asthma attack.

FORMOTEROL ALONE TRANSIENTLY RELAXES RAT AIRWAYS, WHEREAS IN COMBINATION WITH RETIGABINE IT INDUCES GREATER AND MORE SUSTAINED BRONCHODILATION



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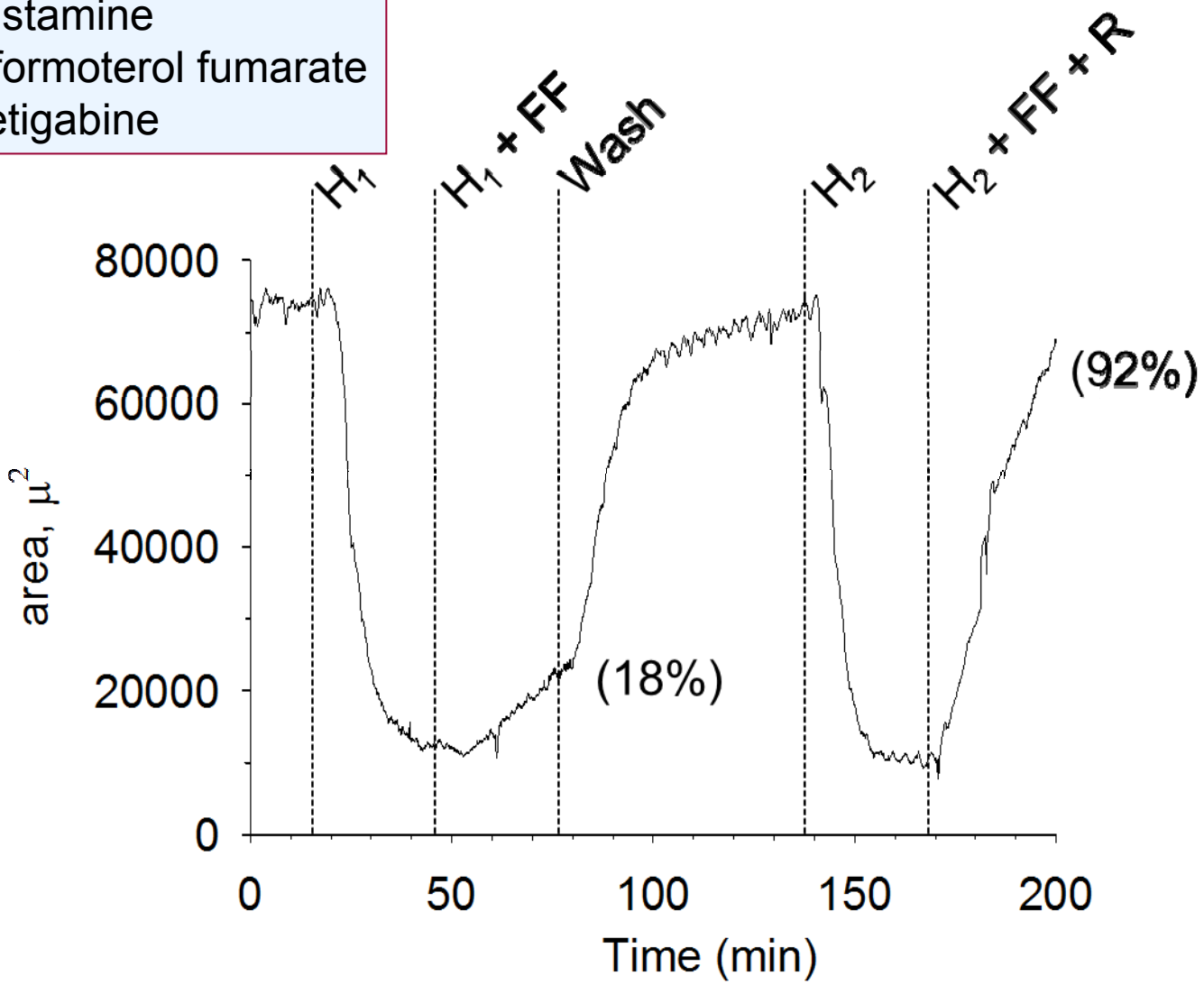
COMBINING RETIGABINE WITH FORMOTEROL IMPROVES RELAXATION OF ASTHMATIC HUMAN AIRWAYS

H = 25 pM histamine
FF = 30 pM formoterol fumarate
R = 10 µM retigabine



CONCLUSIONS

- ✓ KCNQ (Kv7) K^+ channels contribute to stabilization of resting membrane potential and are essential intermediates in vasoconstrictor and bronchoconstrictor signal transduction in vascular and airway smooth muscle, respectively.
- ✓ In intact arteries, drugs that activate vascular KCNQ currents induce vasodilation. Drugs that inhibit KCNQ currents are vasoconstrictors. These effects contribute to changes in arterial blood flow and systemic blood pressure.
- ✓ Drugs currently in clinical use may have unexpected cardiovascular side effects due to previously unrecognized effects on vascular KCNQ channels. To predict these cardiovascular side effects, screening of drugs for actions on vascular KCNQ currents will be required. Some drugs may distinguish among KCNQ channels formed from different subunit combinations.
- ✓ Kv7 channels in airway smooth muscle may be important new targets for treatment of airway diseases such as COPD or asthma. Combination therapy with other bronchorelaxants may improve outcomes.

Acknowledgments:

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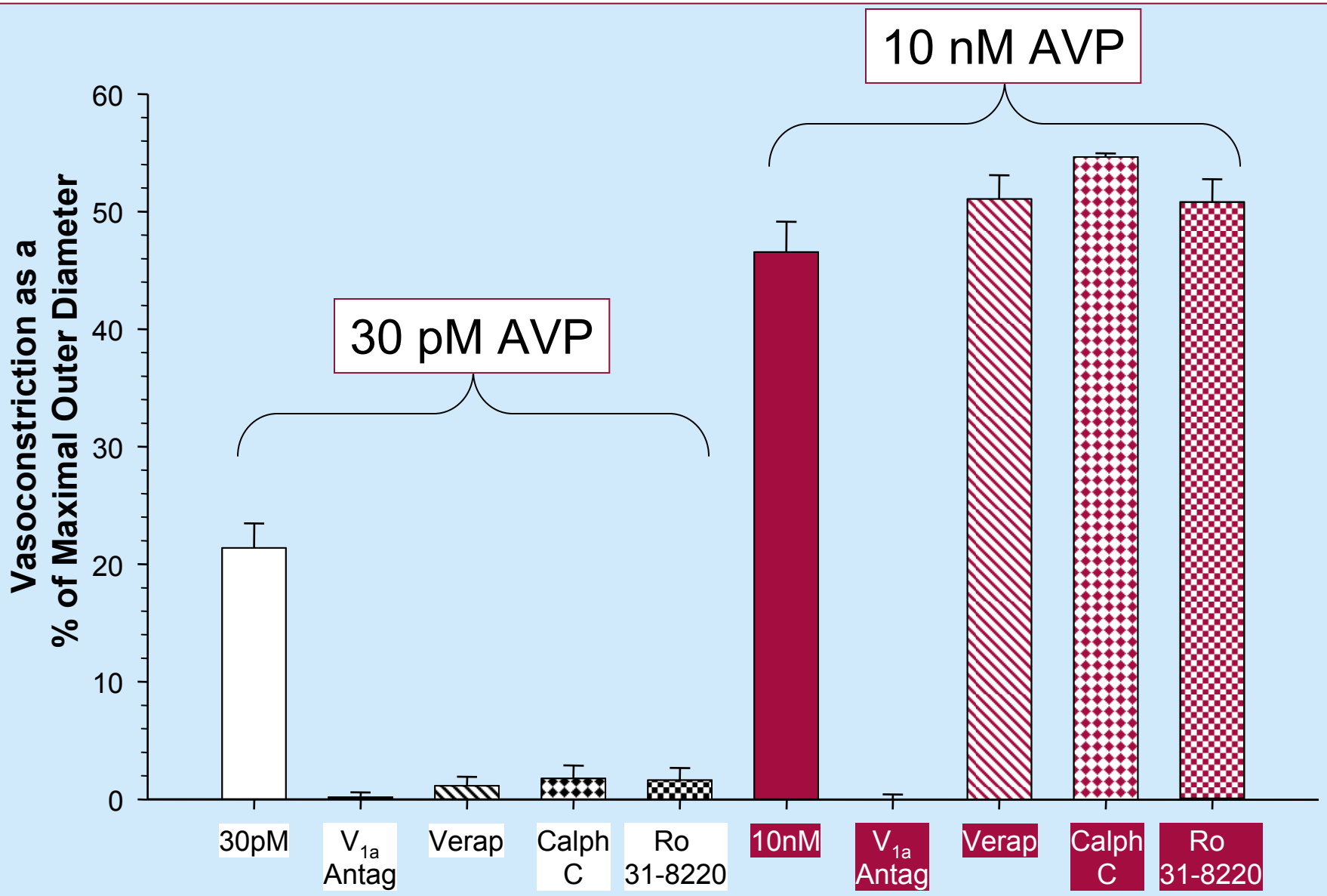
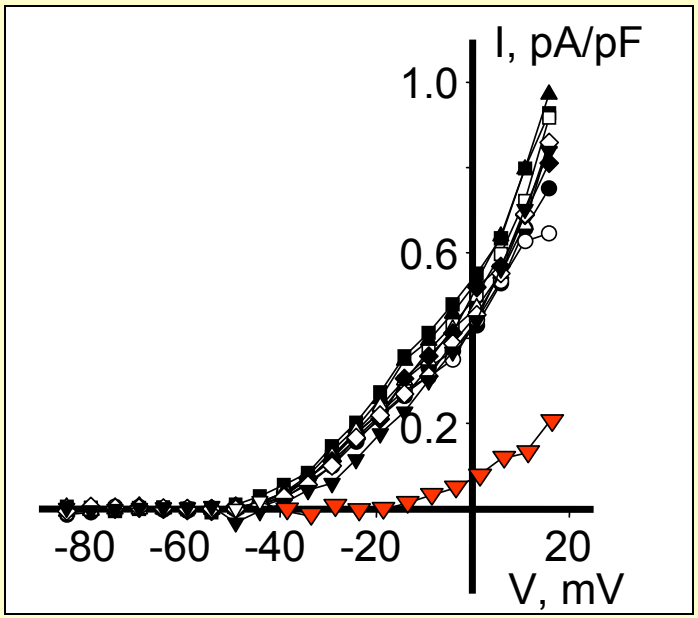
National Heart, Lung, & Blood Institute

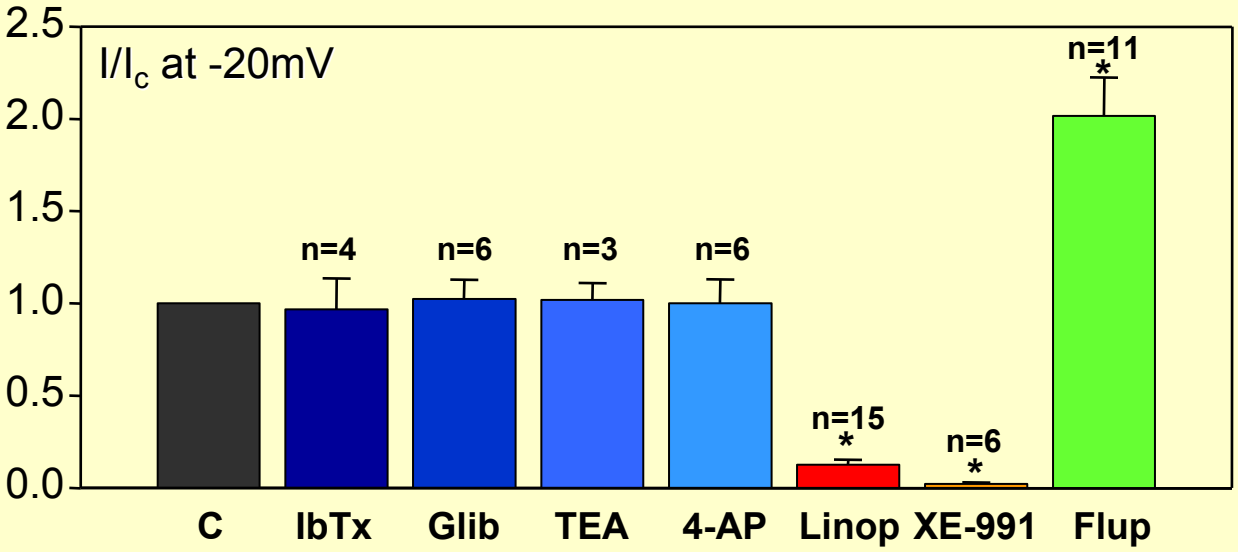
Low [AVP] but not high [AVP] constrictor effects depend on PKC activation and L-type Ca^{2+} channels



KCNQ currents? Isolated K_v currents are not affected by blockers of other types of K^+ channels.



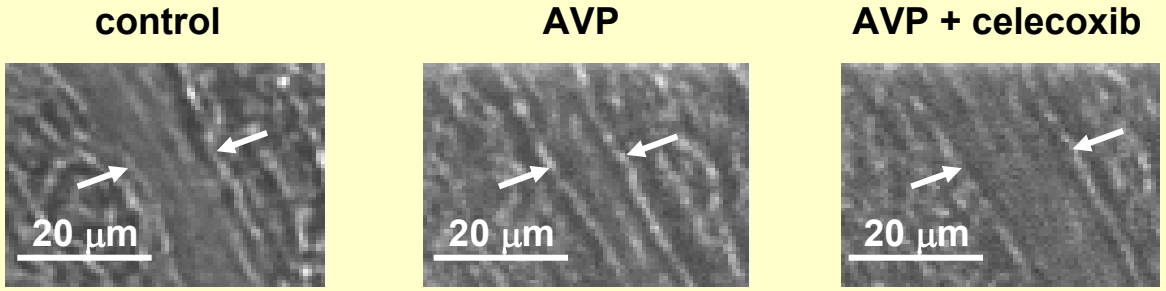
- Control
- 10 µM Glibenclamide
- Washout 1
- 200 nM Iberiotoxin
- Washout 2
- 2 mM Tetraethylammonium
- Washout 3
- 2 mM 4-aminopyridine
- Washout 4
- 10 µM Linopirdine



Mackie *et al.*, *JPET* 2008

Celebrex® dilates mesenteric arterioles and enhances blood flow in vivo

A



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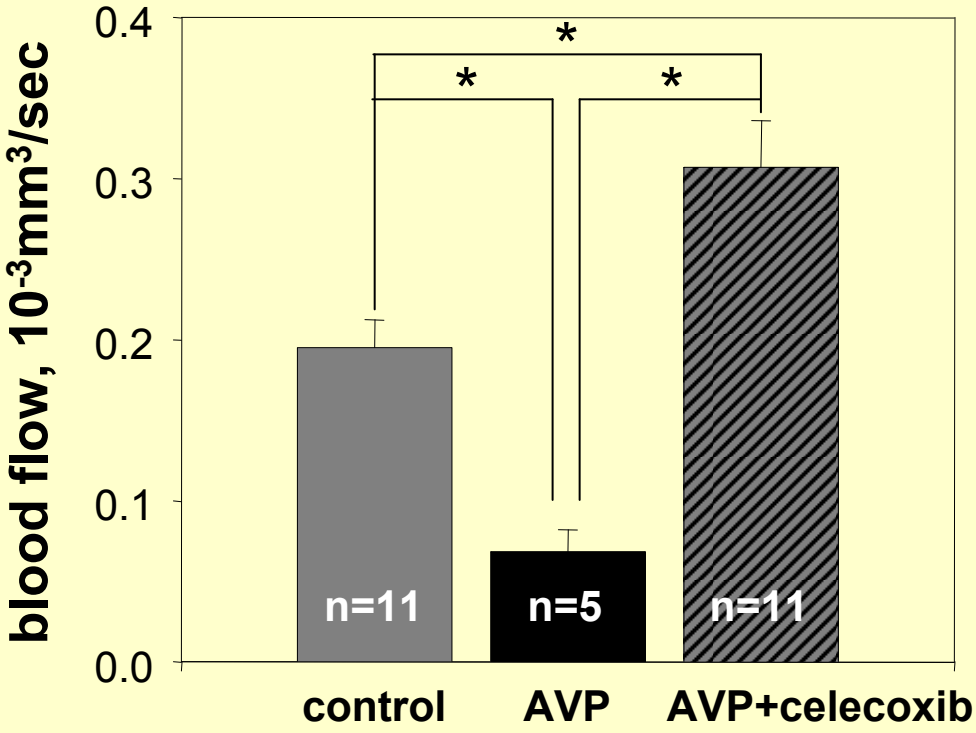
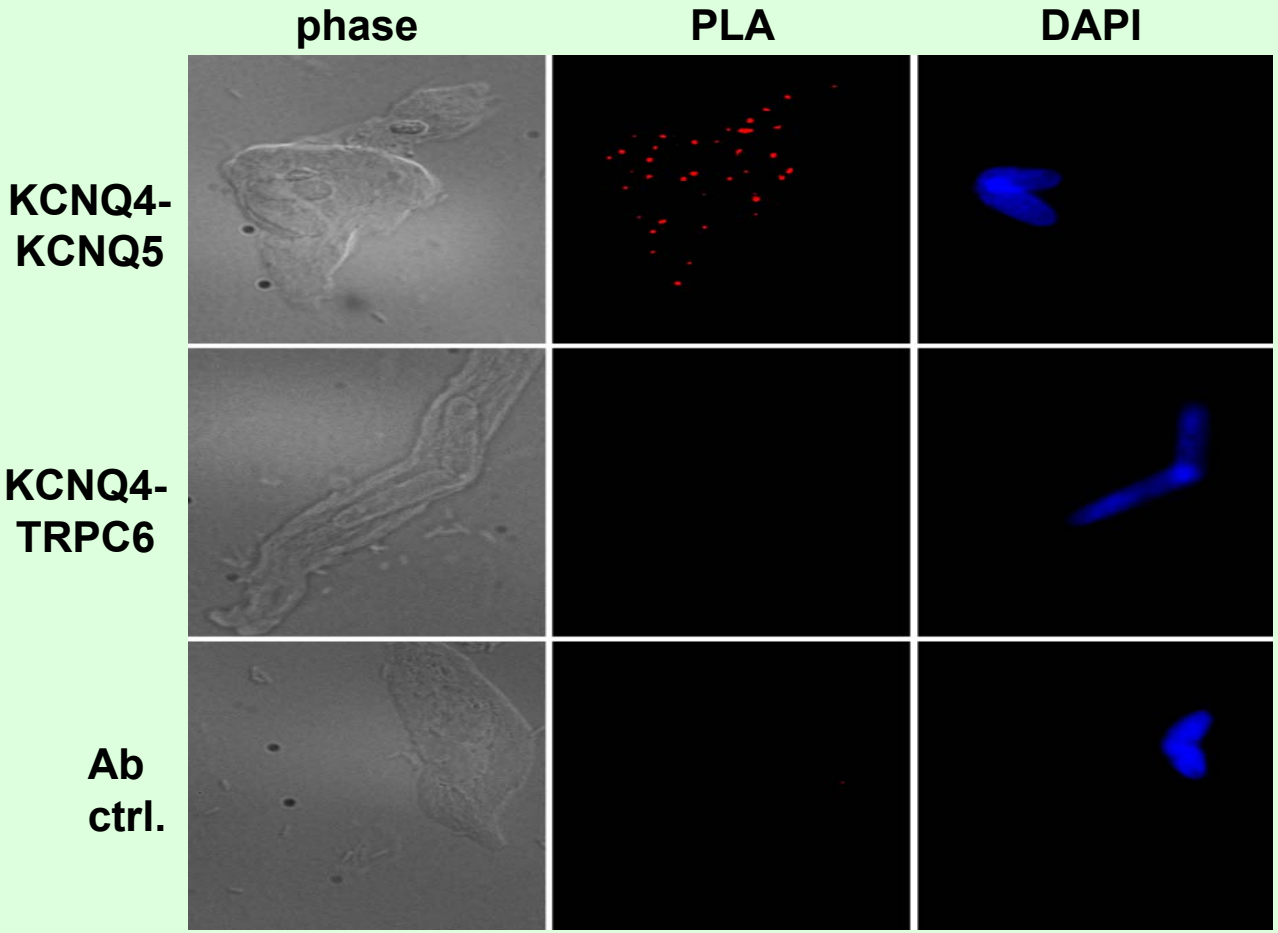
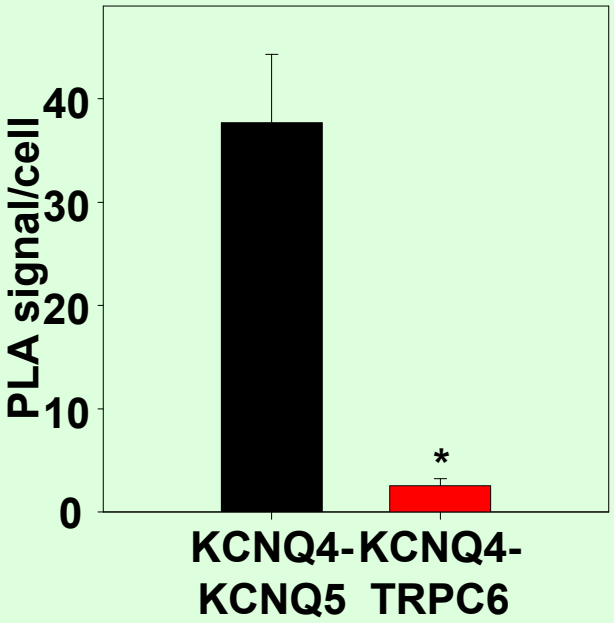
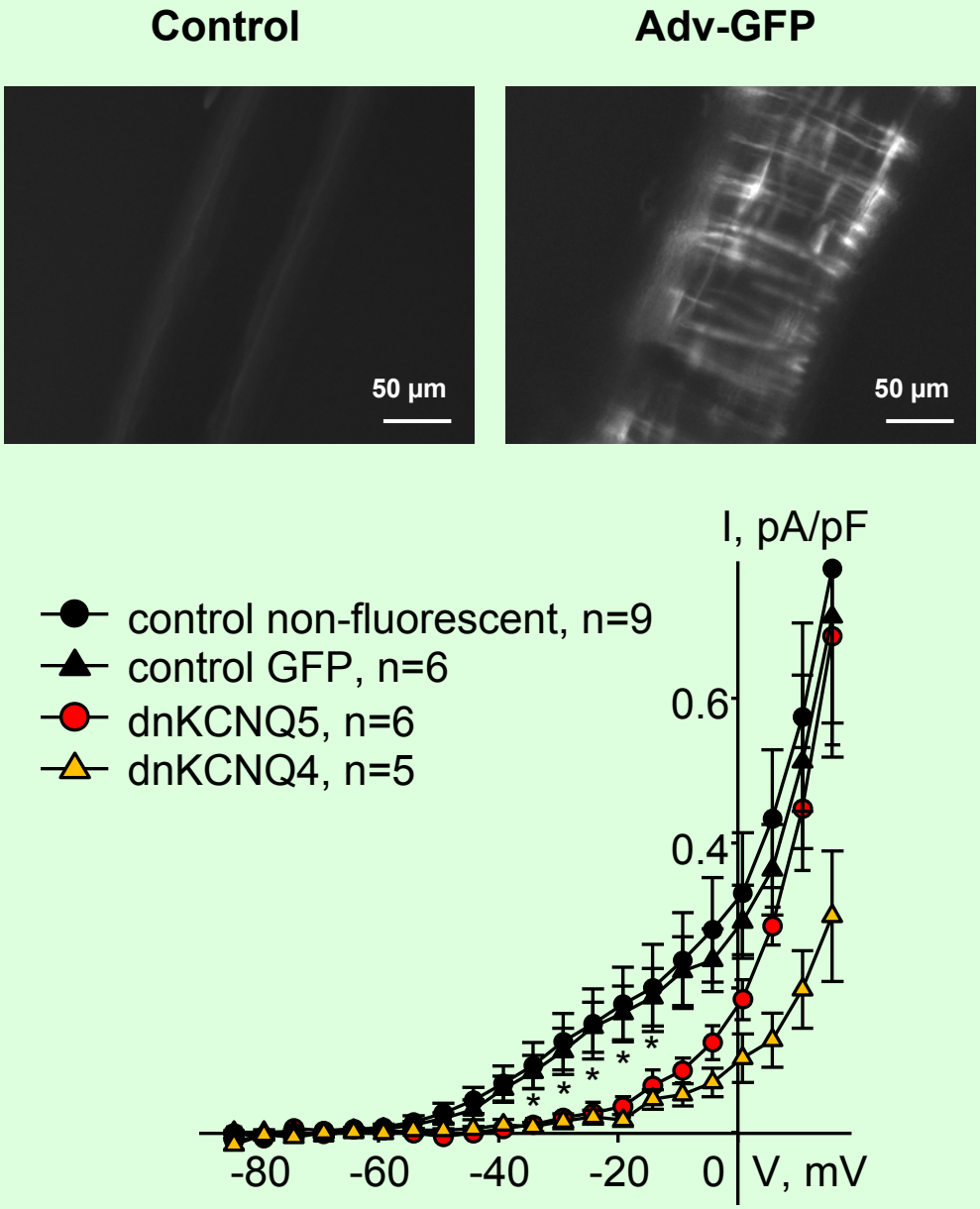


Heteromeric KCNQ4/5 channels in mesenteric artery myocytes.





Brueggemann et al. *JBC* 2014



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