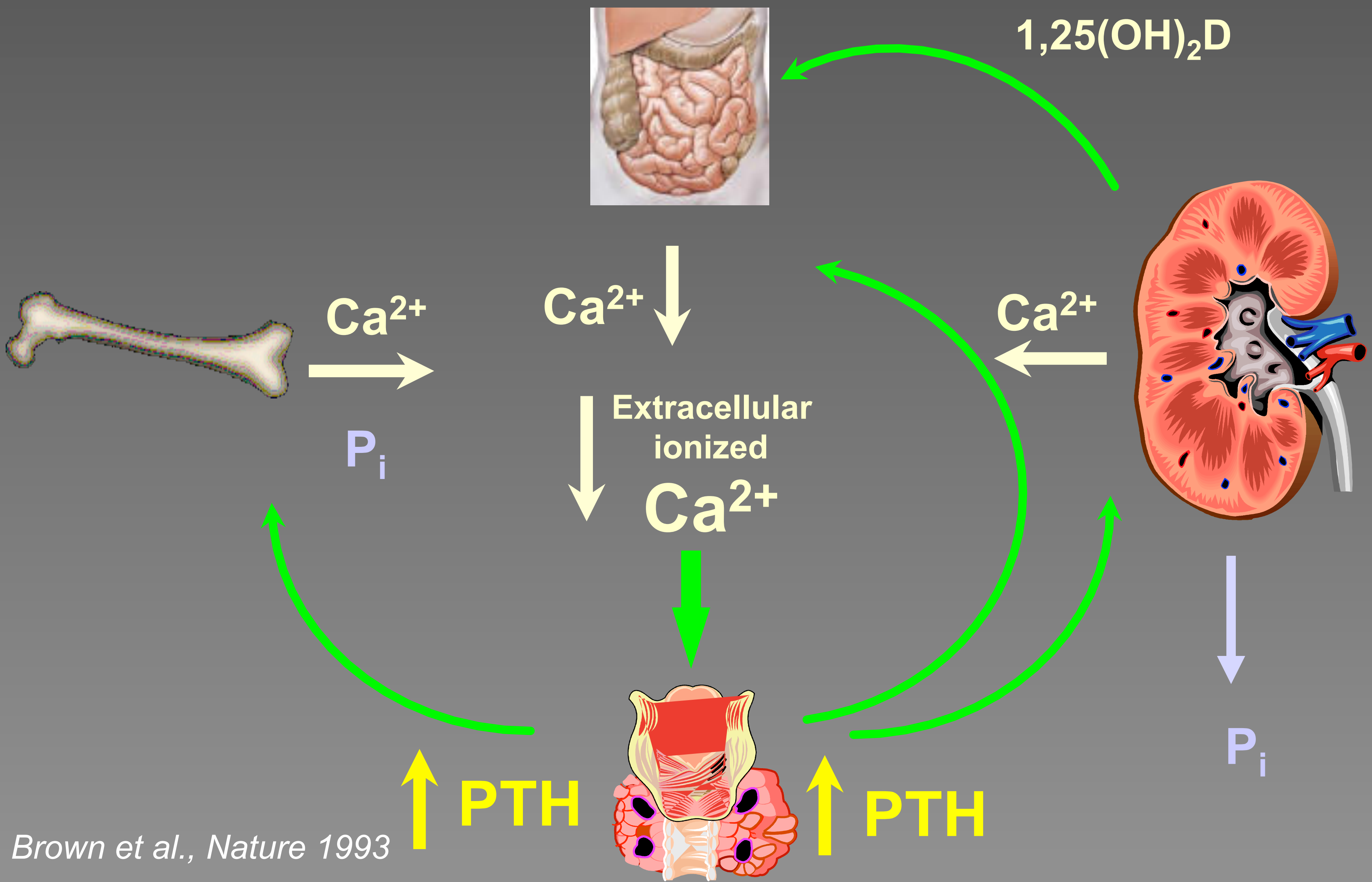
The extracellular calcium-sensing receptor, CaSR, as a nutrient sensor in physiology and disease

Daniela Riccardi, PhD
Cardiff University

Ca^{2+}_o homeostasis:

1. Sensors
2. Effectors

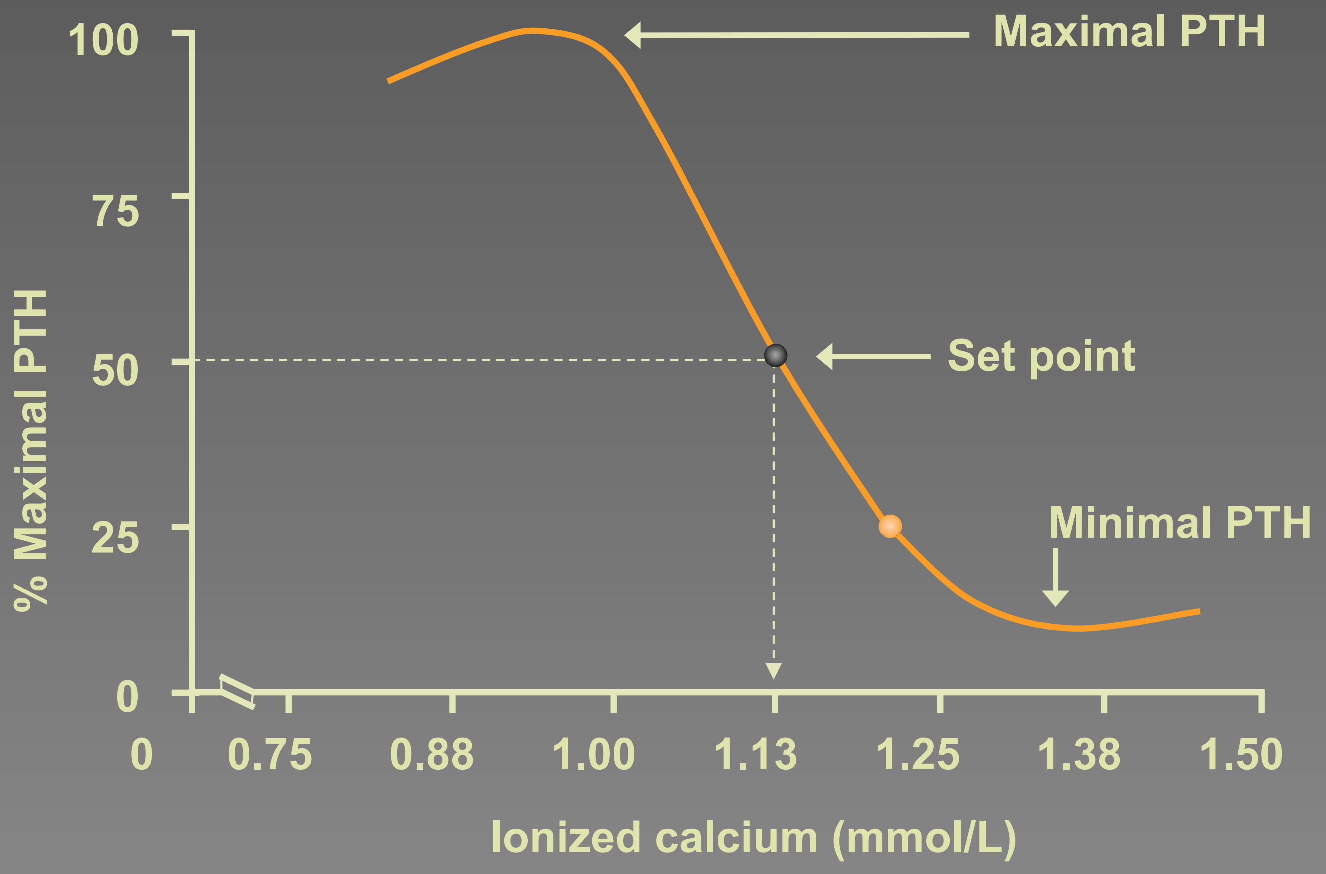


Brown et al., Nature 1993

Relationship between serum Ca^{2+} and PTH

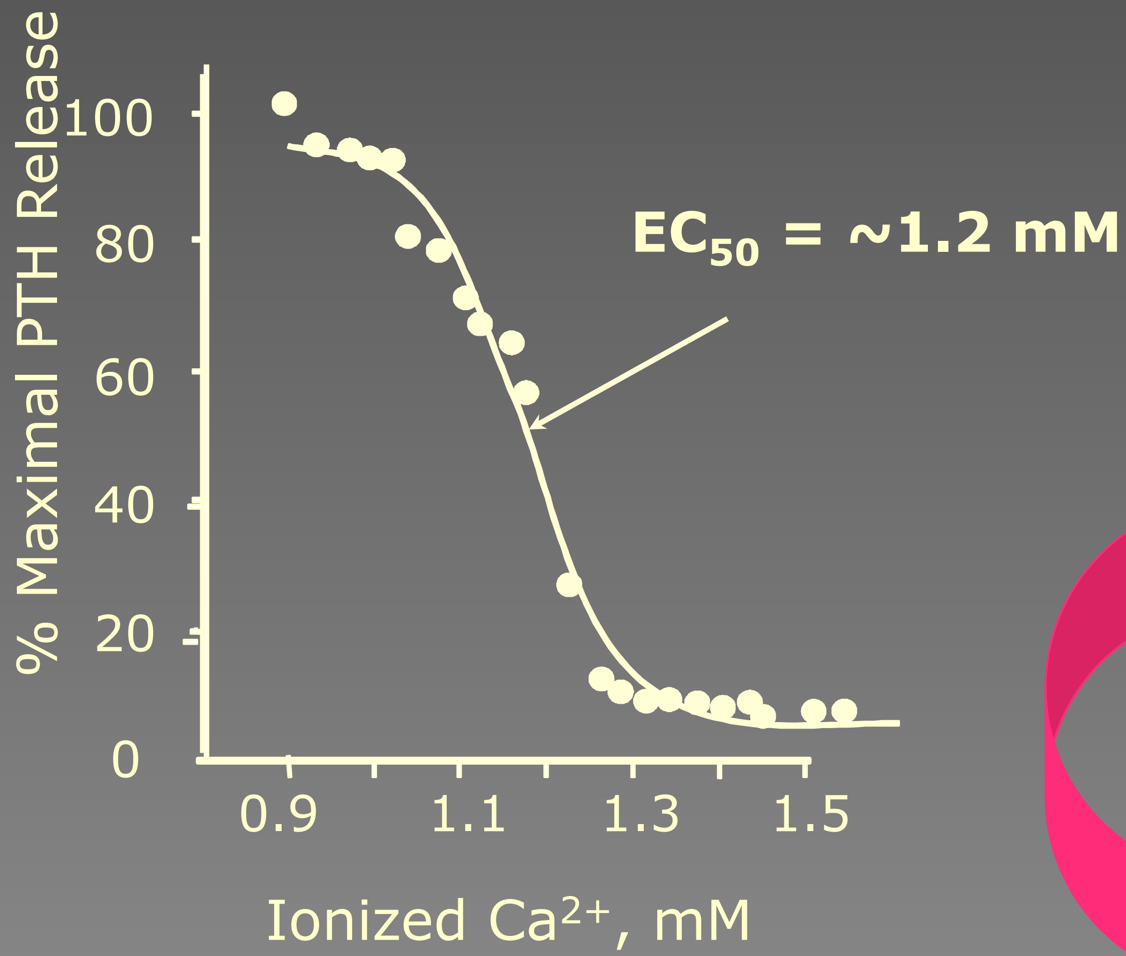
Small changes in serum free ionised calcium (Ca^{2+}) have a dramatic effect on PTH levels.

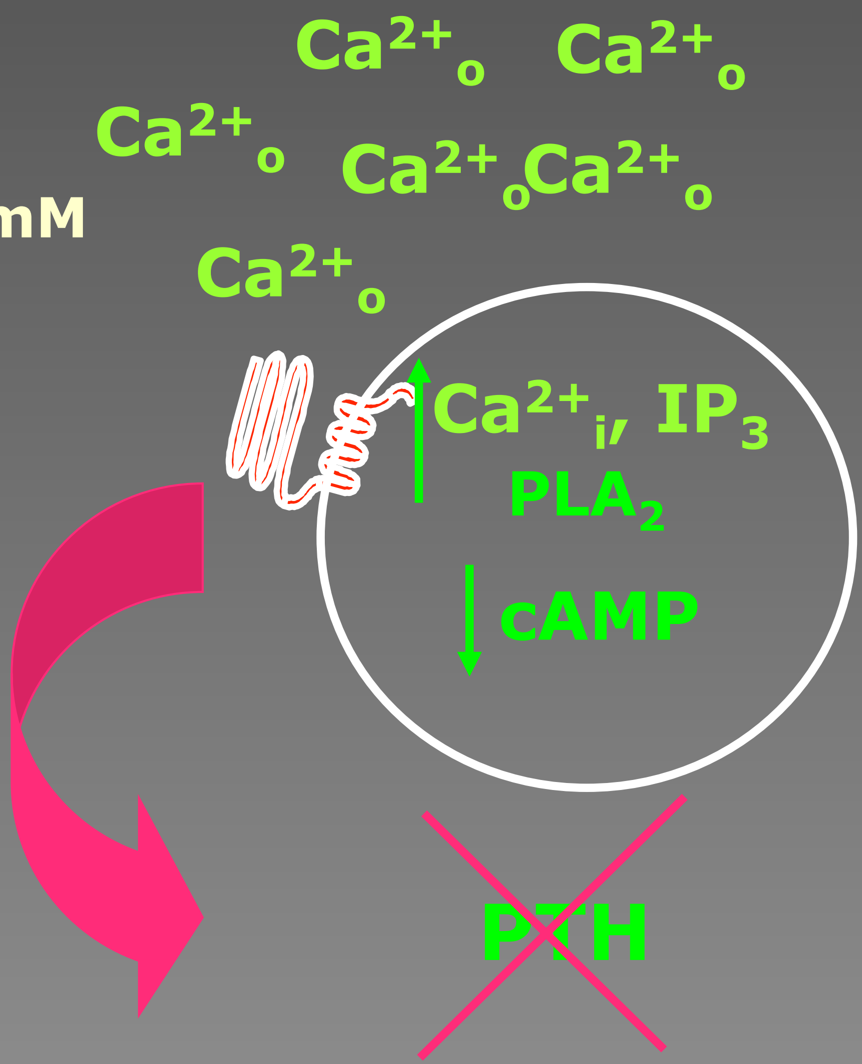
The set point illustrates the sensitivity of the parathyroid glands to serum Ca^{2+}



Adapted, with permission, from Mayer GP *et al.* *Endocrinology* 1978;102:1036–42
Felsenfeld AJ, Llach F. *Kidney Int* 1993;43:771–89

Extracellular calcium-sensing in the parathyroid glands





Brown et al., 1991

The extracellular calcium-sensing receptor, CaSR

CaSR belongs to Group C GPCRs (tasteR, pheromoneR, mGluRs, $GABA_B$, GPRC6a/5.24)

ORTHOSTERIC MODULATORS:

spermine

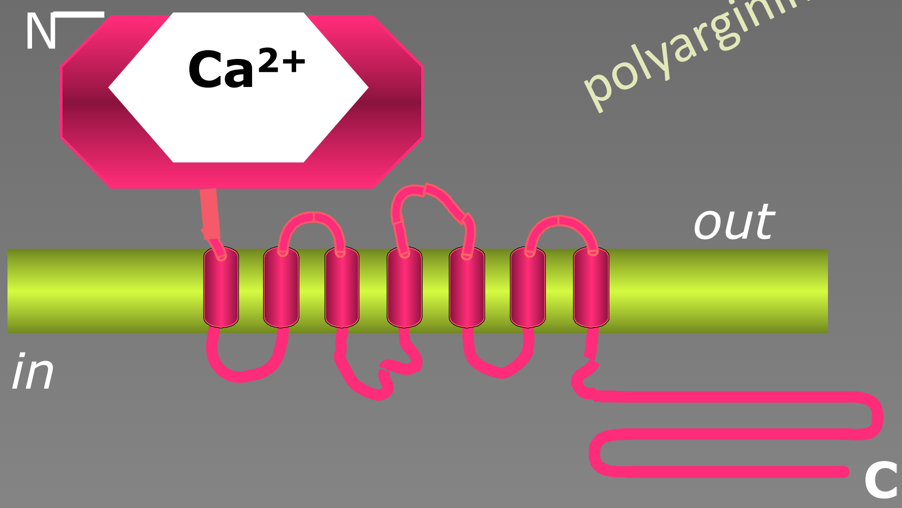
Gd^{3+}

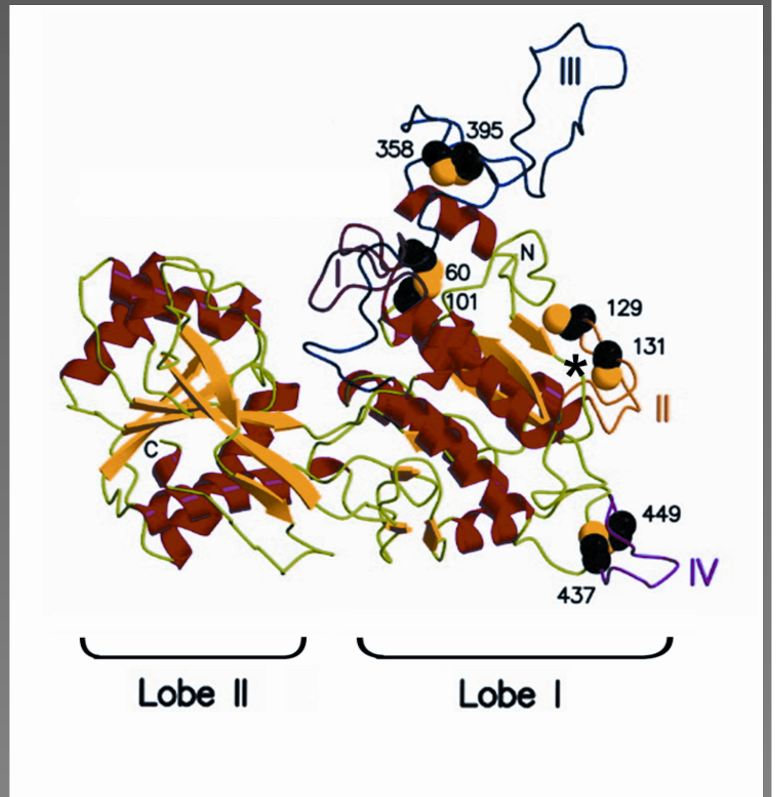
gentamicin

Mg^{2+}

ß-amyloid peptides

polyarginine





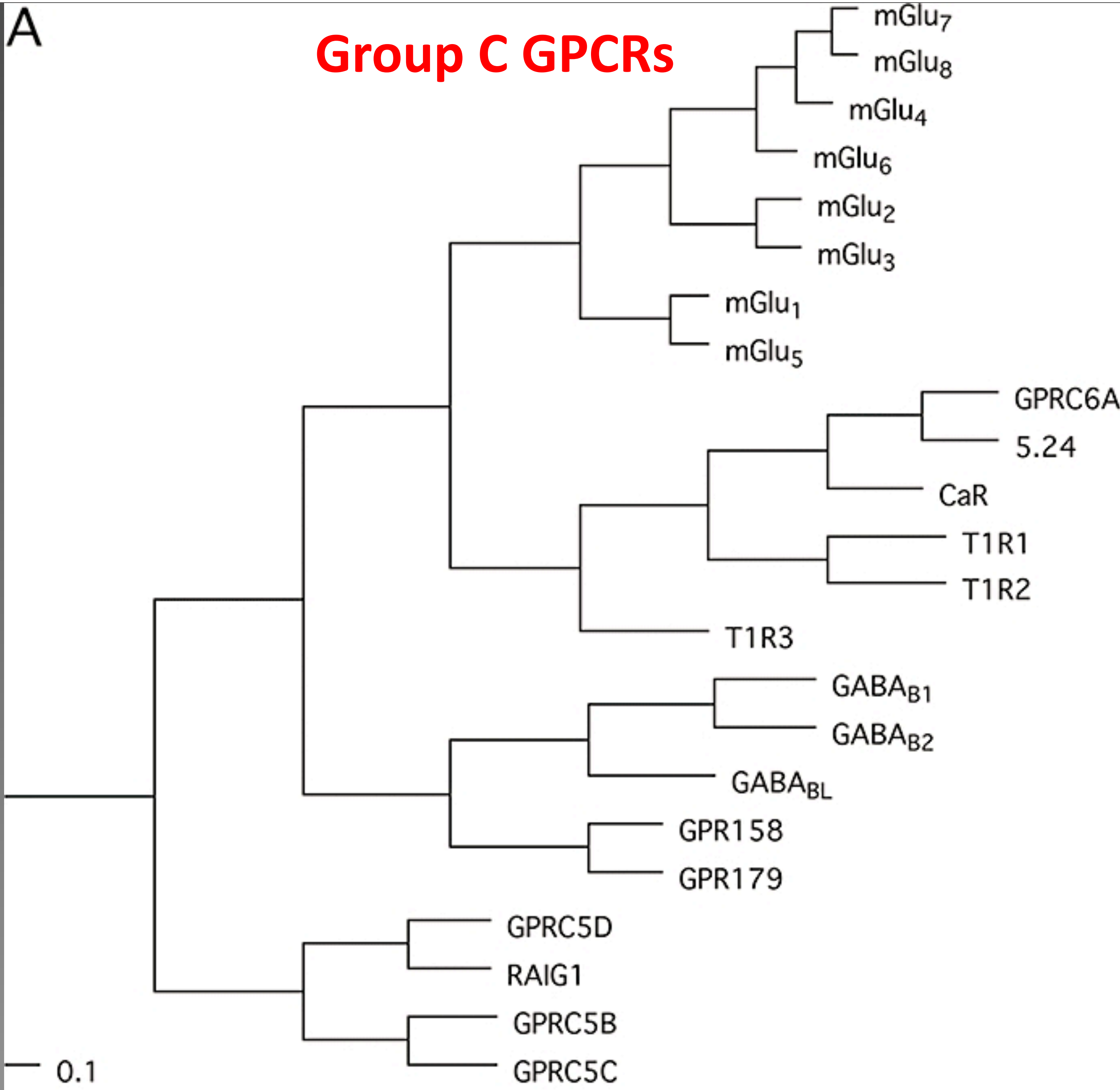
ALLOSTERIC MODULATORS:

L-aromatic aa, Ionic strength, GSH analogs

+ve: calcimimetics (Sensipar/Mimpara)

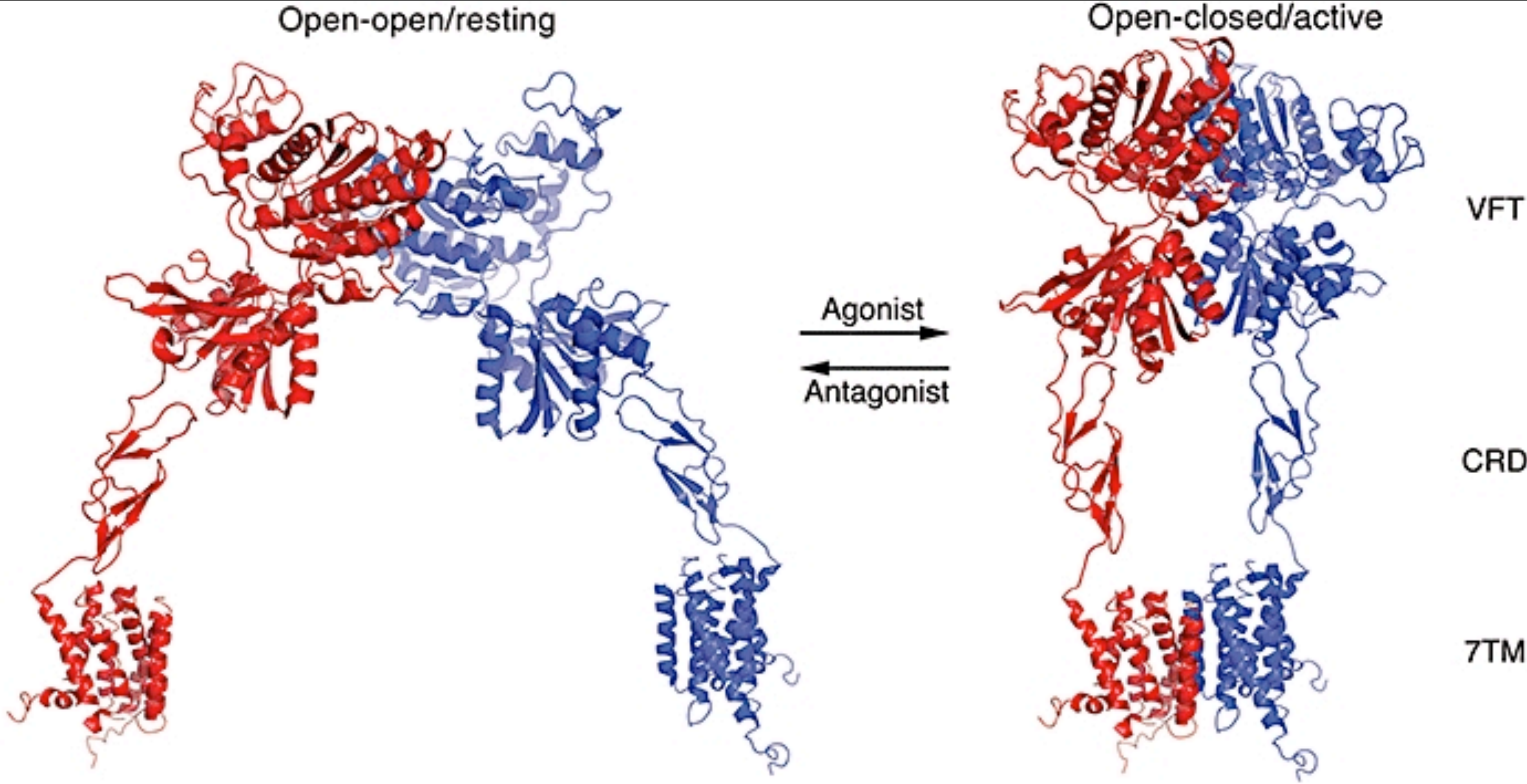
-ve: calcilytics (ie ronacaleret)

Group C GPCRs



Wellendorph and Bräuner-Osborne (2009)

Model of a dimeric group C GPCR

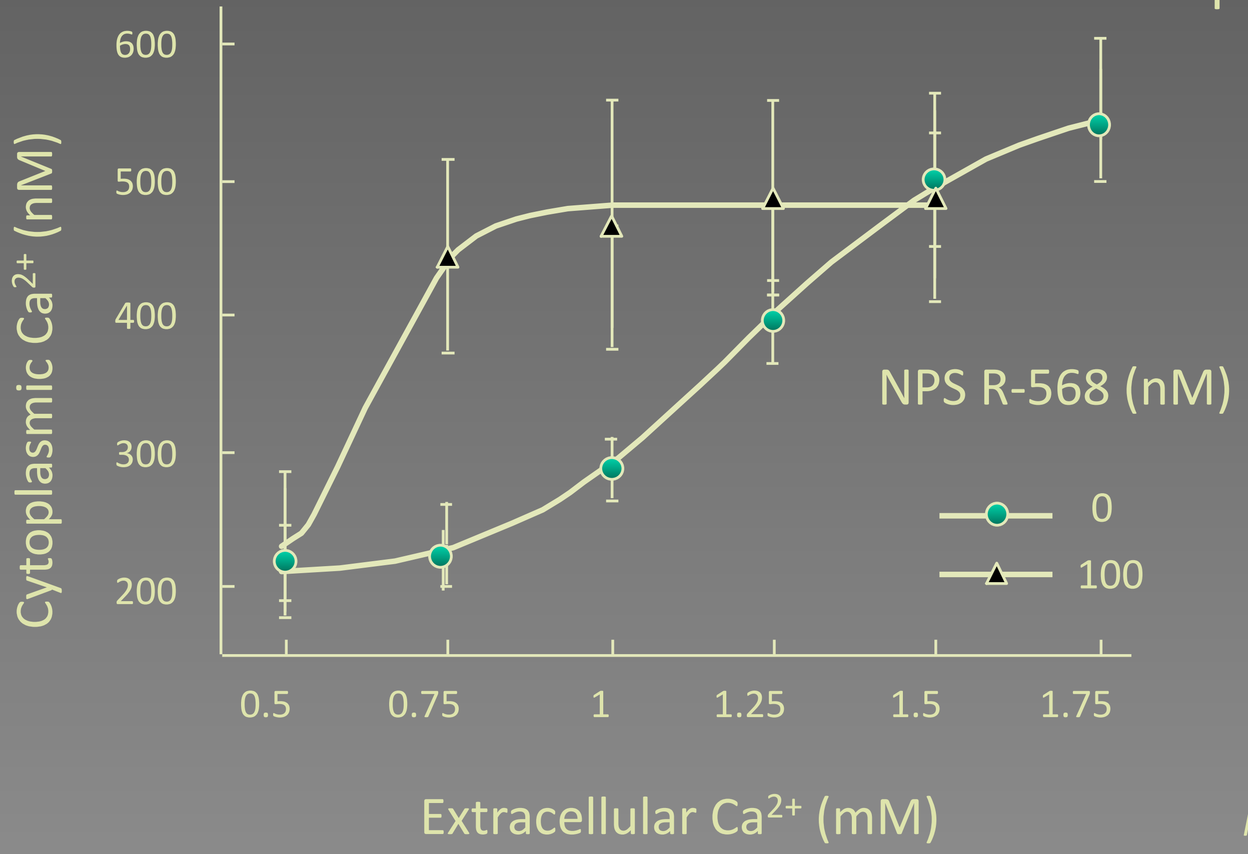


Wellendorph and Bräuner-Osborne (2009)

CaSR positive allosteric modulators or "calcimimetics"

Low MW phenylaklylamines. Ineffective in the absence of extracellular Ca^{2+} (Nemeth *et al*., 1998); stereoselective (R > S), CaSR-specific (*i.e*., do not affect other GPCRs)

•*In vitro* studies show a leftward shift in the Ca^{2+} dose-response at the CaSR.



Nemeth et al, 1998

• R-568 and AMG073 decrease PTH and Ca^{2+} levels in a dose-dependent manner (2003)

The CaSR as a drug target

Calcimimetic compounds

FDA approval in 2004 for the treatment of:

1. Hyperparathyroidism secondary to kidney failure
2. Parathyroid carcinoma

Off-label: Correct PTH-dependent hypercalcemia following renal transplantation; Li-induced hyperparathyroidism; X-linked hypophosphatemia & oncogenic osteomalacia

Calcilytic compounds

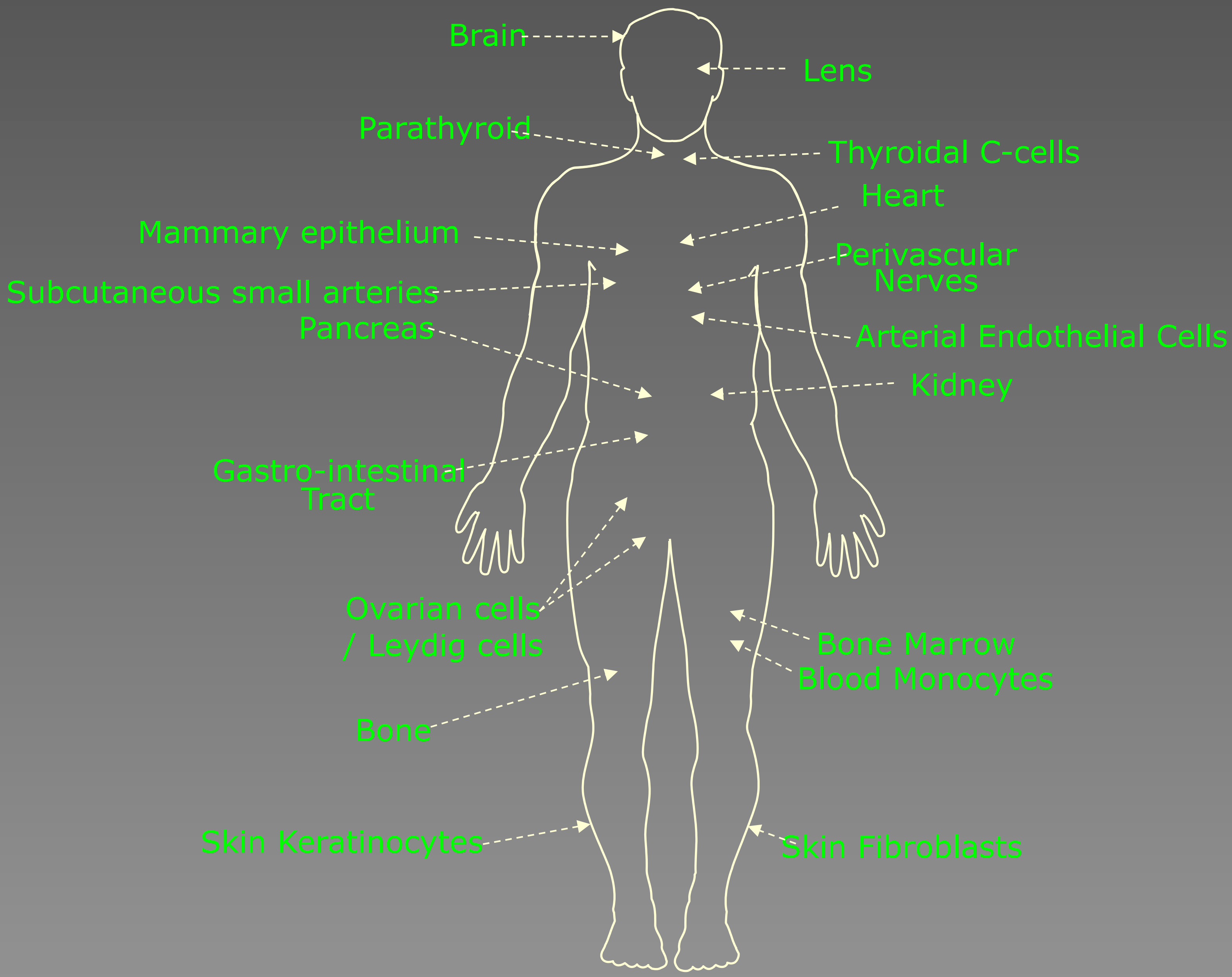
Enhance endogenous PTH secretion, inducing an oscillating hormone concentration profile (a known anabolic stimulus).

Increase bone mass in osteoporosis?

Diseases associated with abnormalities of the extracellular calcium-sensing receptor (CaSR)

CaSR Abnormality and Disease	Biological effect
Loss-of-function CaSR mutations	
Familial hypocalciuric hypercalcemia	Hypocalciuria
NSHPT	Hypocalciuria
Gain-of-function CaSR mutations	
Autosomal Dominant hypocalcemia	Hypercalciuria
Bartter syndrome type V	Hypokalemia/hyperreninemia
CaSR auto-antibodies	
Inactivating (AHH)	Hypocalciuria
Activating (Acquired hypoparathyroidism)	Hypercalciuria
CaSR polymorphisms	
990G	Hypercalciuria
986A, 990C, 1011G (Haplotypes)	Increased risk of stones

The CaSR is expressed in regions not involved in Ca^{2+}_o homeostasis



CaSR in the GI tract

Organ	Cell	Effect	Reference
Stomach			
	G cells	Gastrin secretion	[55]
		Cell growth	
	Parietal cells	Acid secretion (H^+-K^+ ATPase)	[56]
Intestine			
Duodenum	Enteric nervous system cells	Gut motility	[35]
	I cells	CCK secretion	[47]
	K cells	GIP secretion	[49]
	L cells	GLP-1 and PYY secretion	[49]
	Enteric nervous system cells	Inhibition of fluid secretion	[57]
Colon	Colonocytes	Inhibition of cell proliferation	[58,59]
		Stimulation of cell differentiation	[35]
		Inhibition of ion/fluid secretion	[35]

1. Taste receptor for both Ca^{2+} and protein
2. Amino acid sensor for the release of dietary hormones

MUTATIONS OF THE CaSR GENE RESULT IN INHERITED DISORDERS OF CALCIUM METABOLISM

Familial Hypocalciuric (benign) Hypercalcemia, FHH:

- Haploinsufficiency
- PTH levels inappropriately normal
- Renal concentrating ability normal
- Mild hypercalcemia and hypermagnesemia
- Parathyroidectomy ineffective in normalizing hypercalcemia

Phenotype: Generally asymptomatic, occasionally pancreatitis and gall stones

FROM DISEASE TO FUNCTION: CaSR IN THE PANCREAS

Pancreatitis has been identified as a complication of FHH in a distinct subgroup of patients

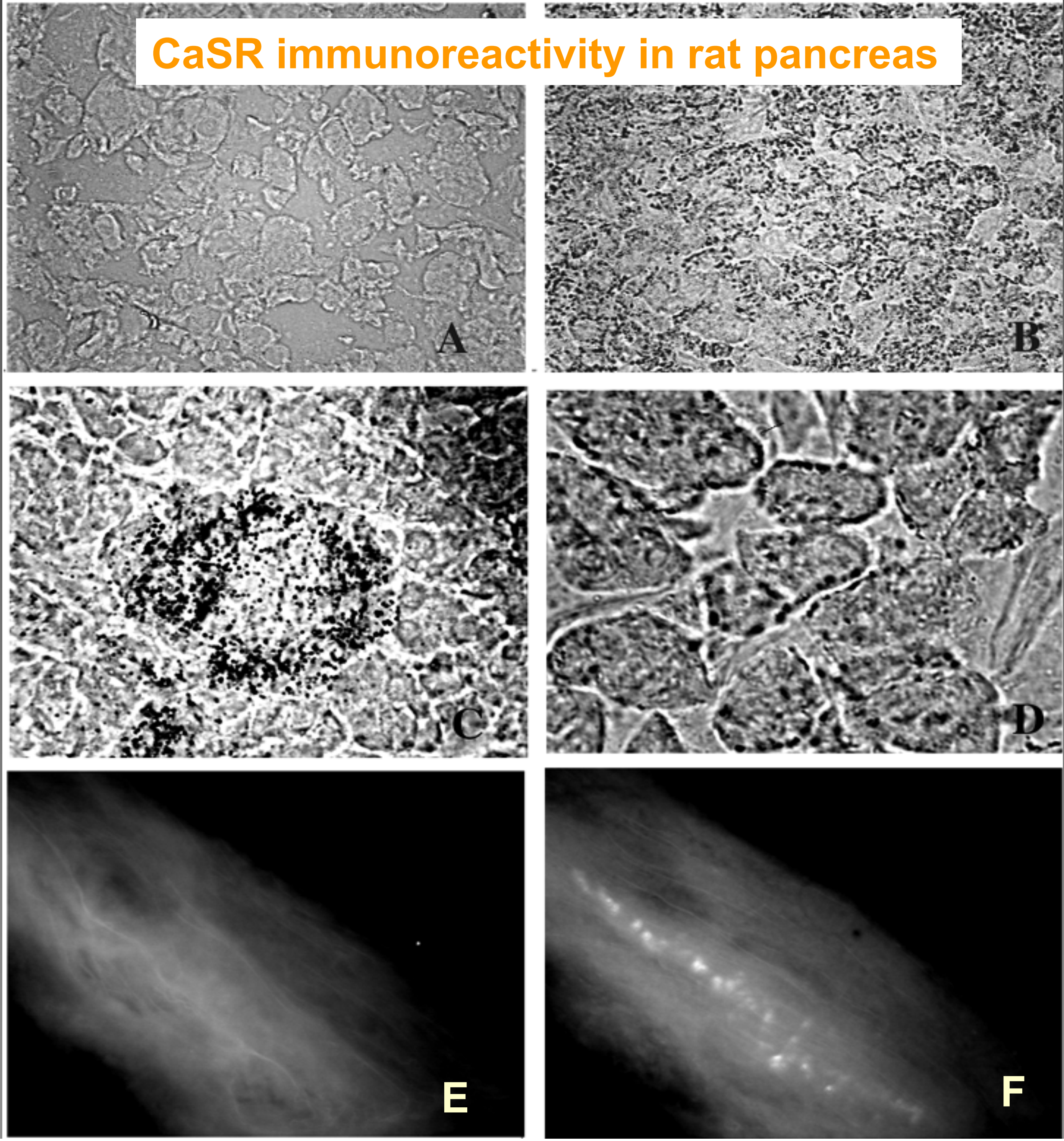
Hypercalcemia is a known etiologic factor for pancreatic disease

In the human pancreatic juice: $[HCO_3^-]$: 140 mM
$[Ca^{2+}]$: 1-1.2 mM

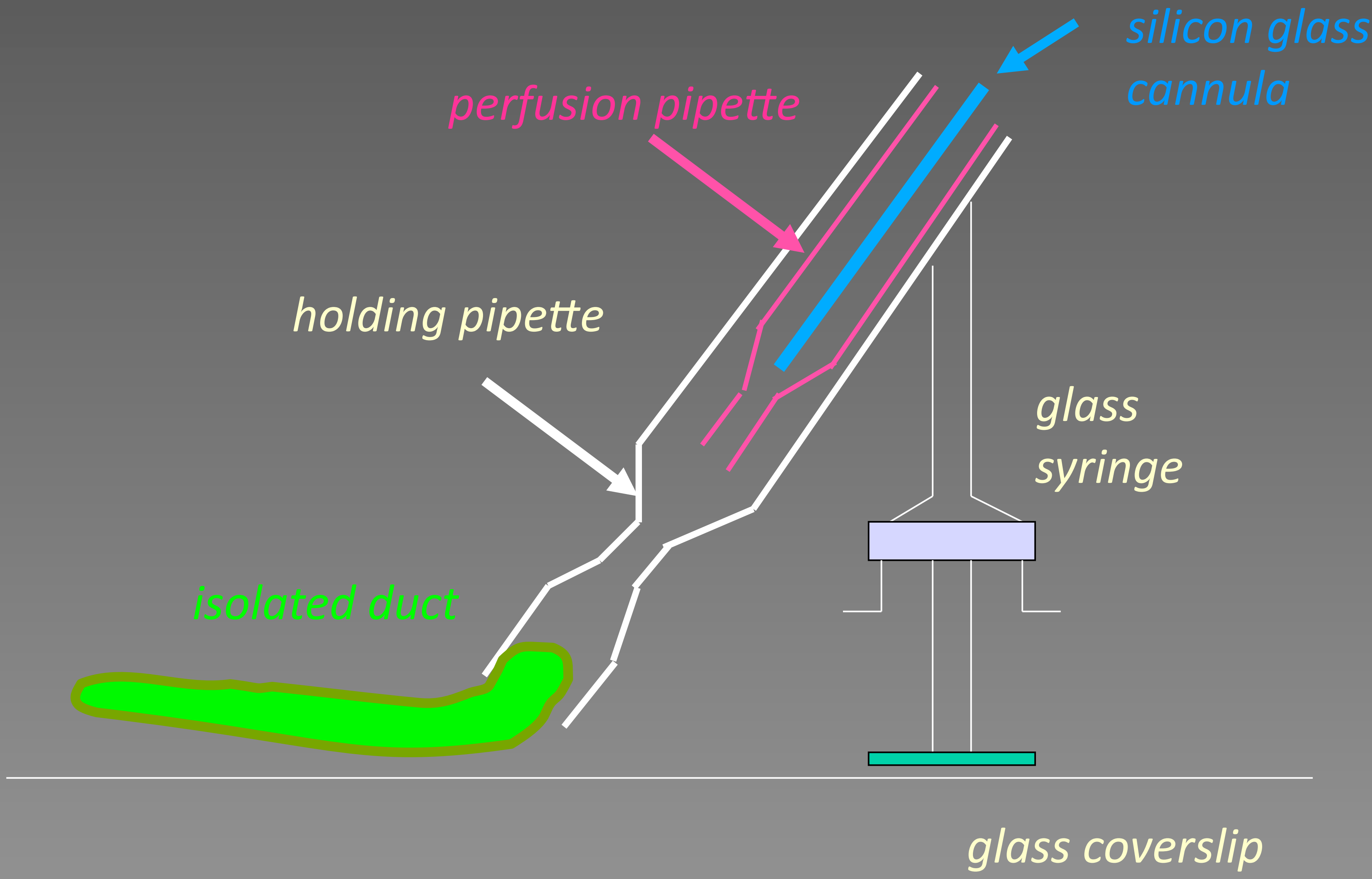
Despite this, the incidence of pancreatic stone formation is low

Which are the homeostatic responses that reduce the lithogenic potential?

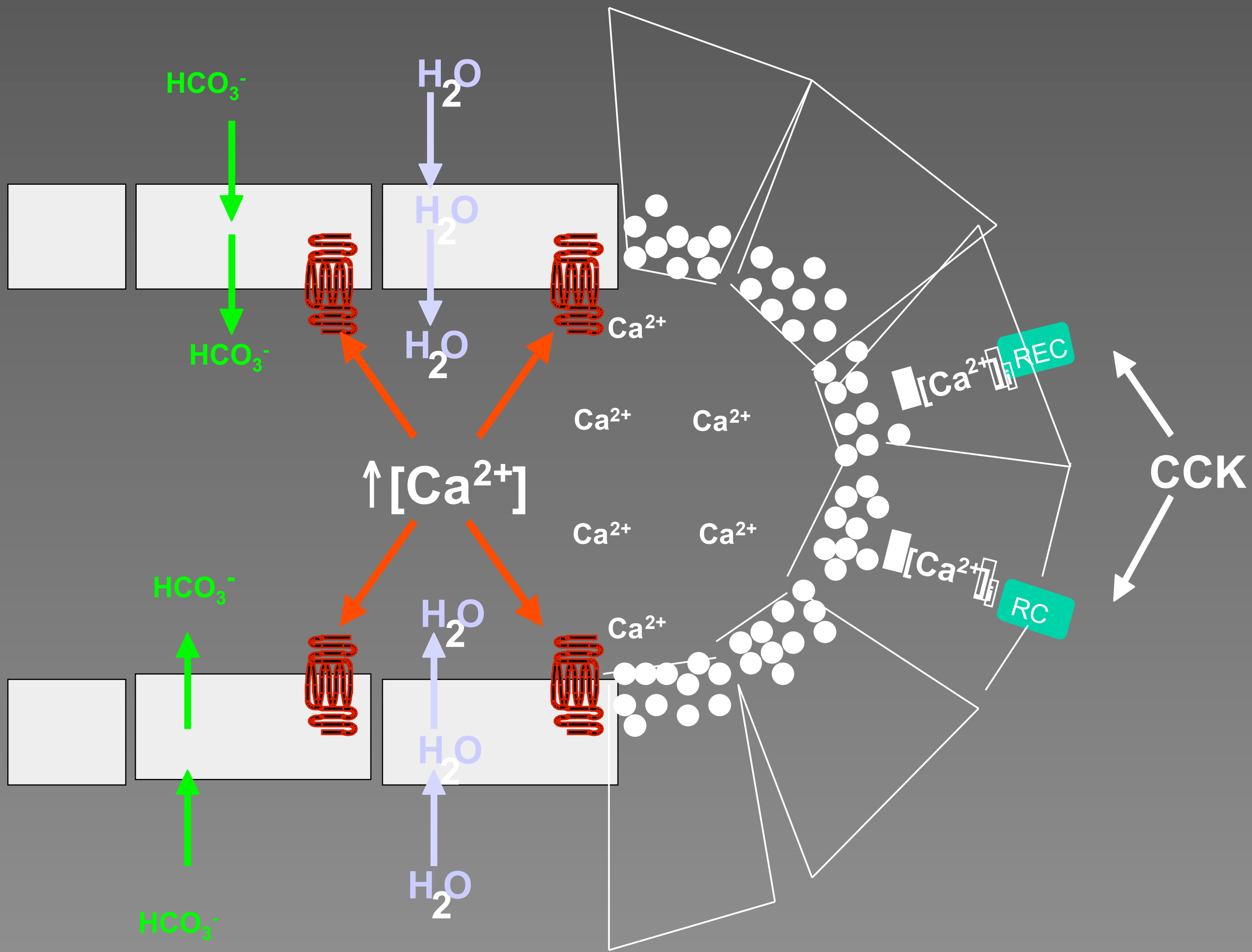
CaSR immunoreactivity in rat pancreas



MICROPERFUSION OF ISOLATED PANCREATIC DUCTS

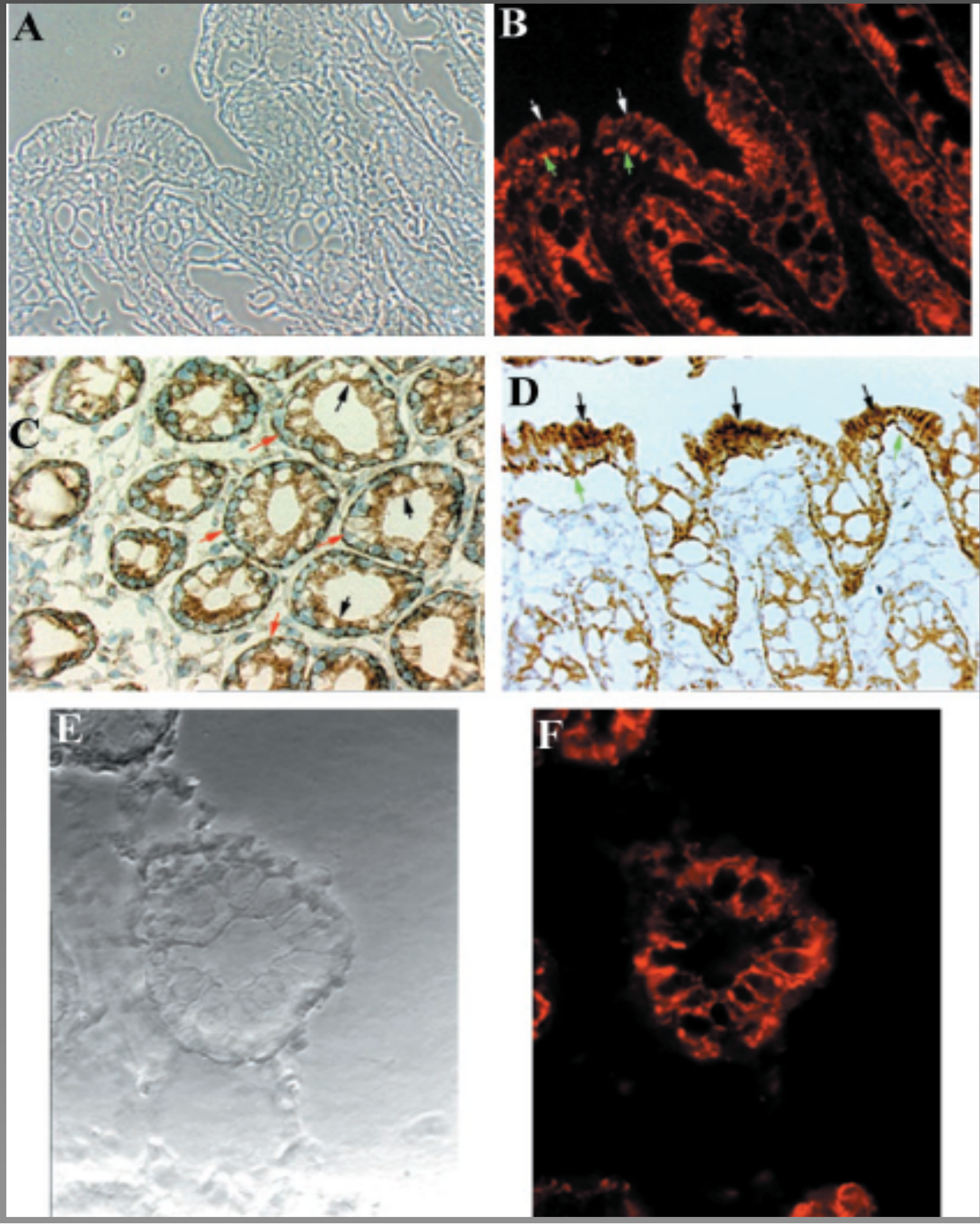


MODEL FOR CaSR FUNCTION IN PANCREATIC DUCTS



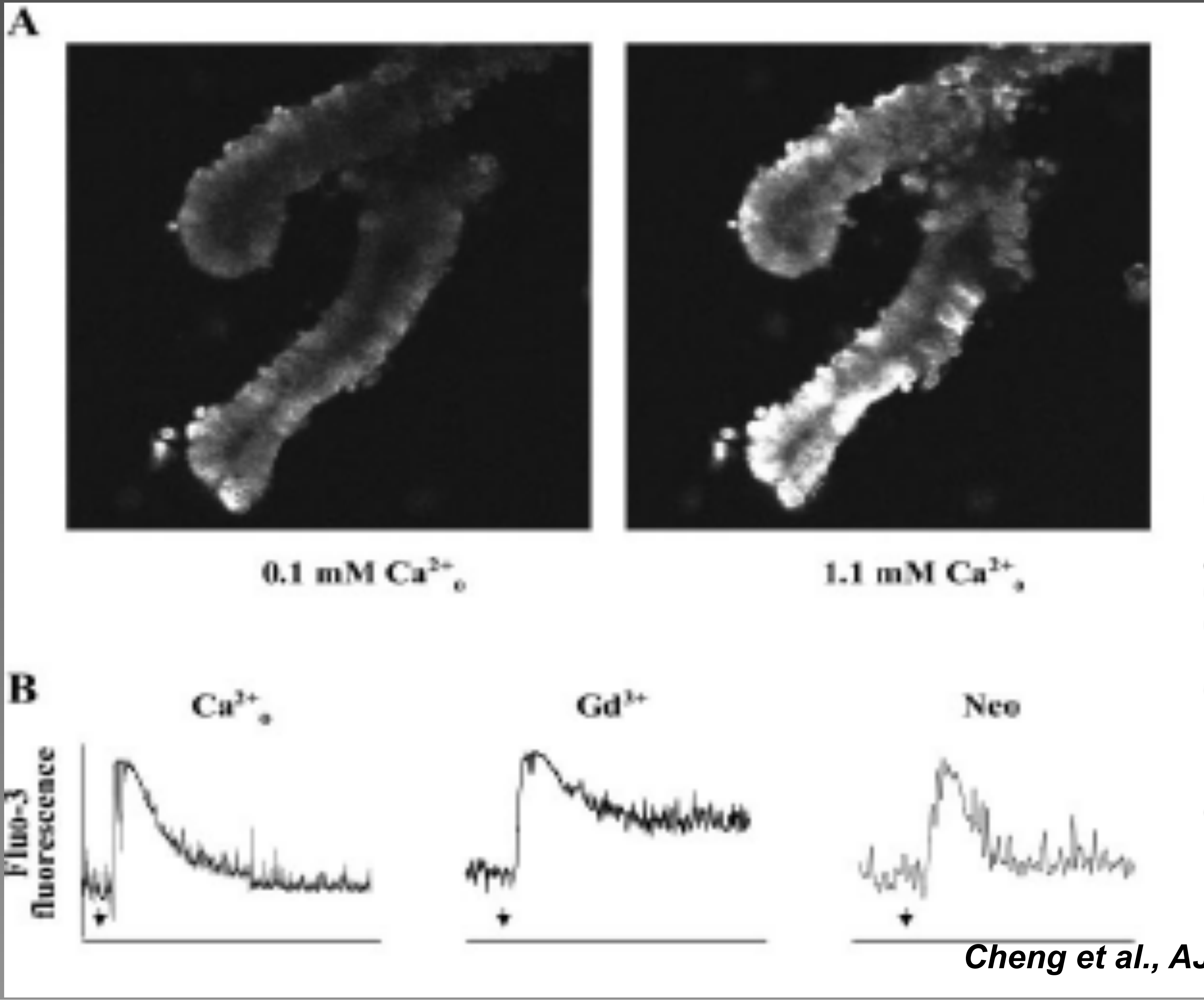
Bruce et al., J Biol Chem 1999

CaSR immunoreactivity in rat (A-D) and human (E,F) intestine



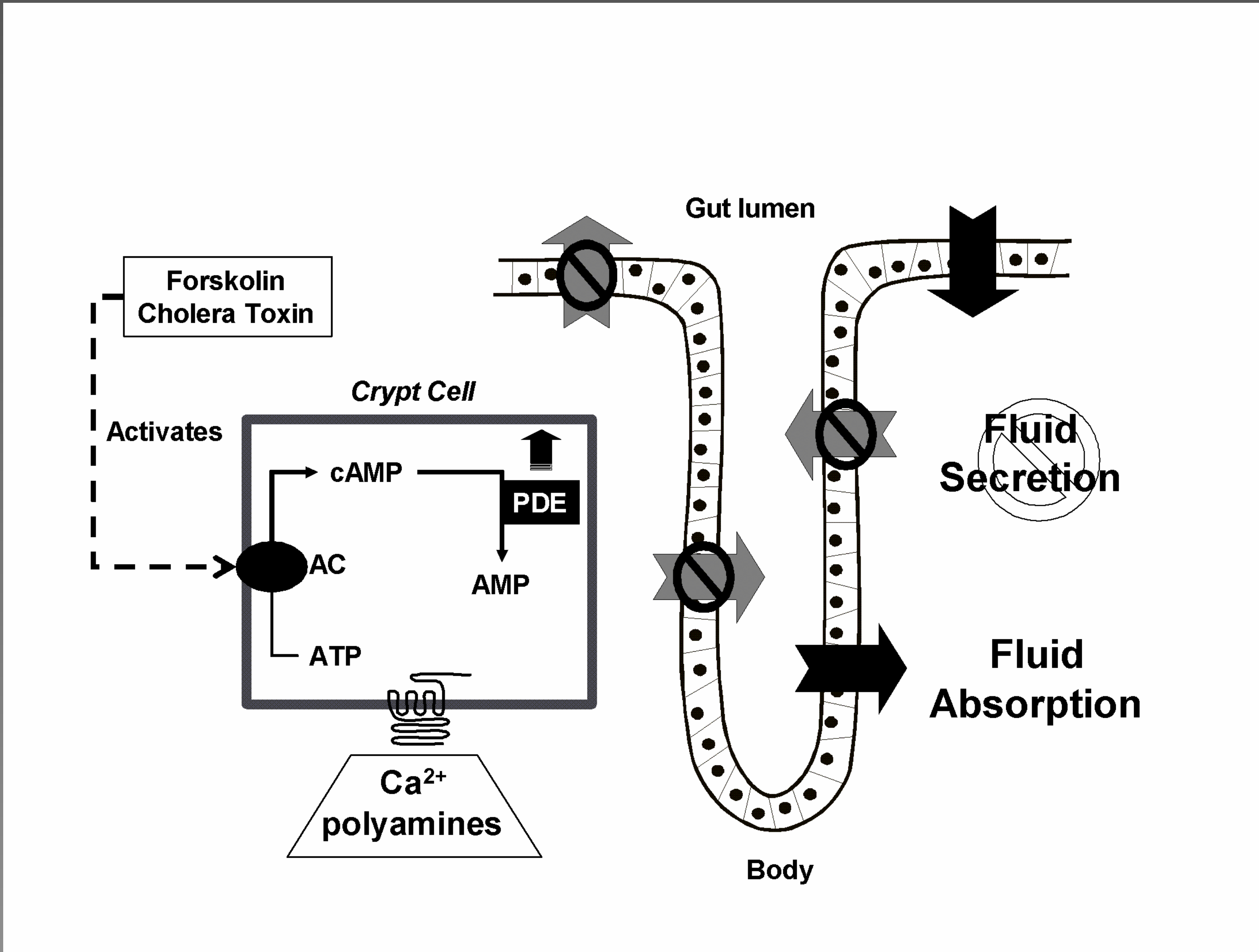
Cheng et al., AJP 2002

Responses to CaSR agonists in epithelial cells of rat colon



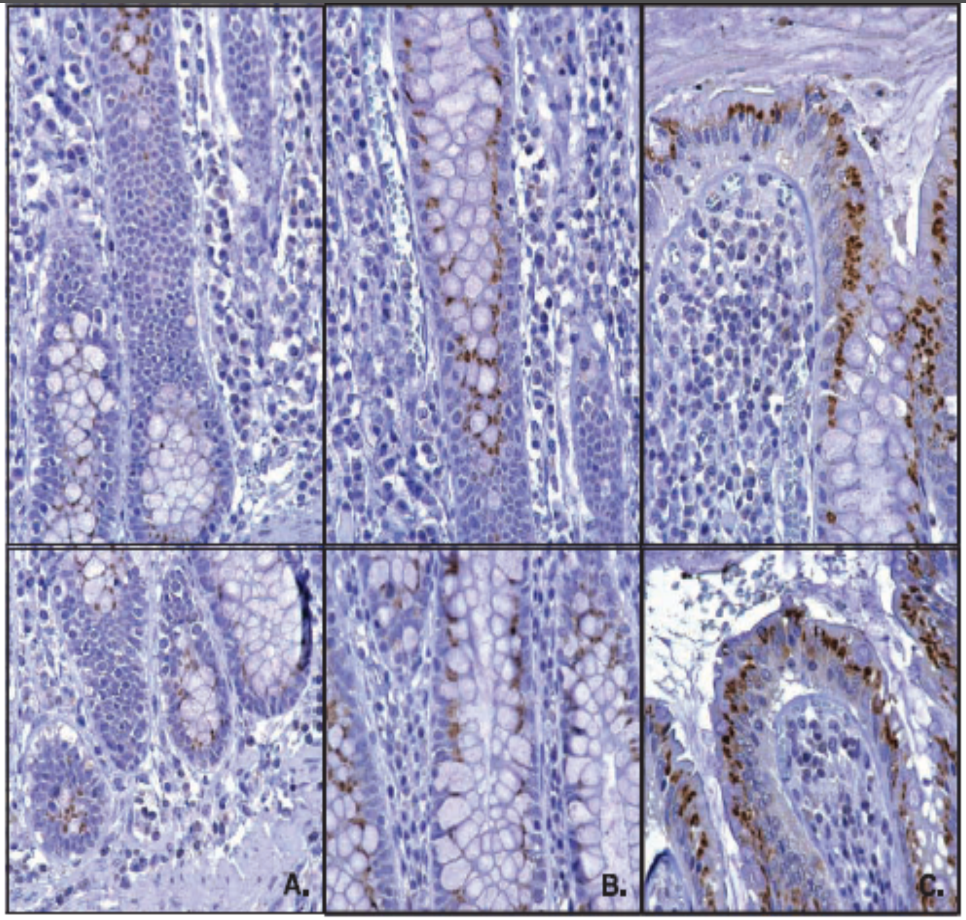
Cheng et al., AJP 2002

Proposed mechanism for CaSR modulation of fluid secretion by the mammalian colonic crypt

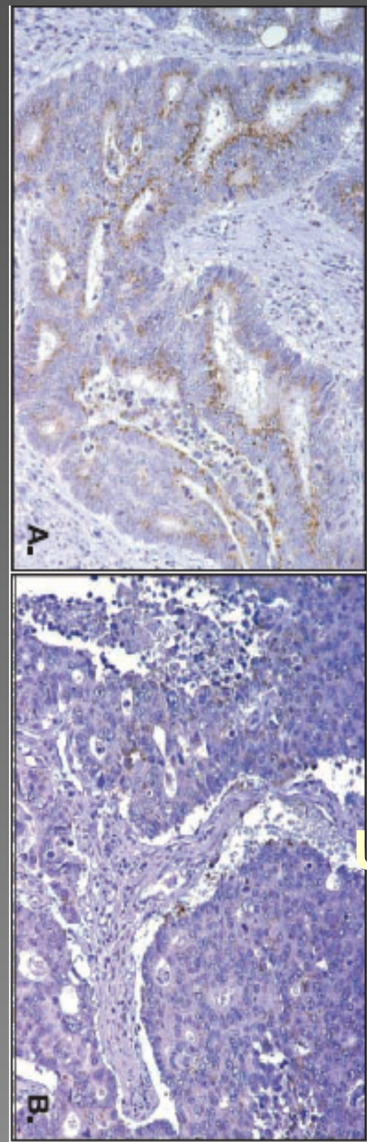


Hebert et al., Cell Calcium 2004

The CaSR, dietary calcium, and colon cancer

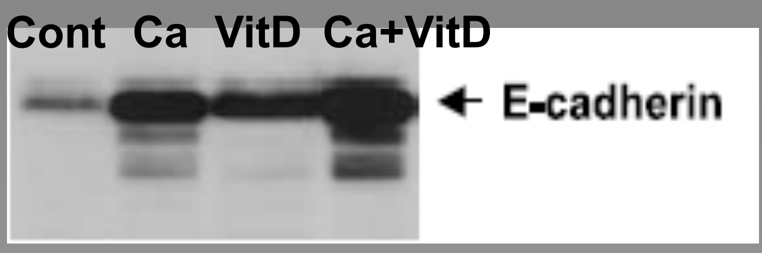


Normal colonic crypt epithelium



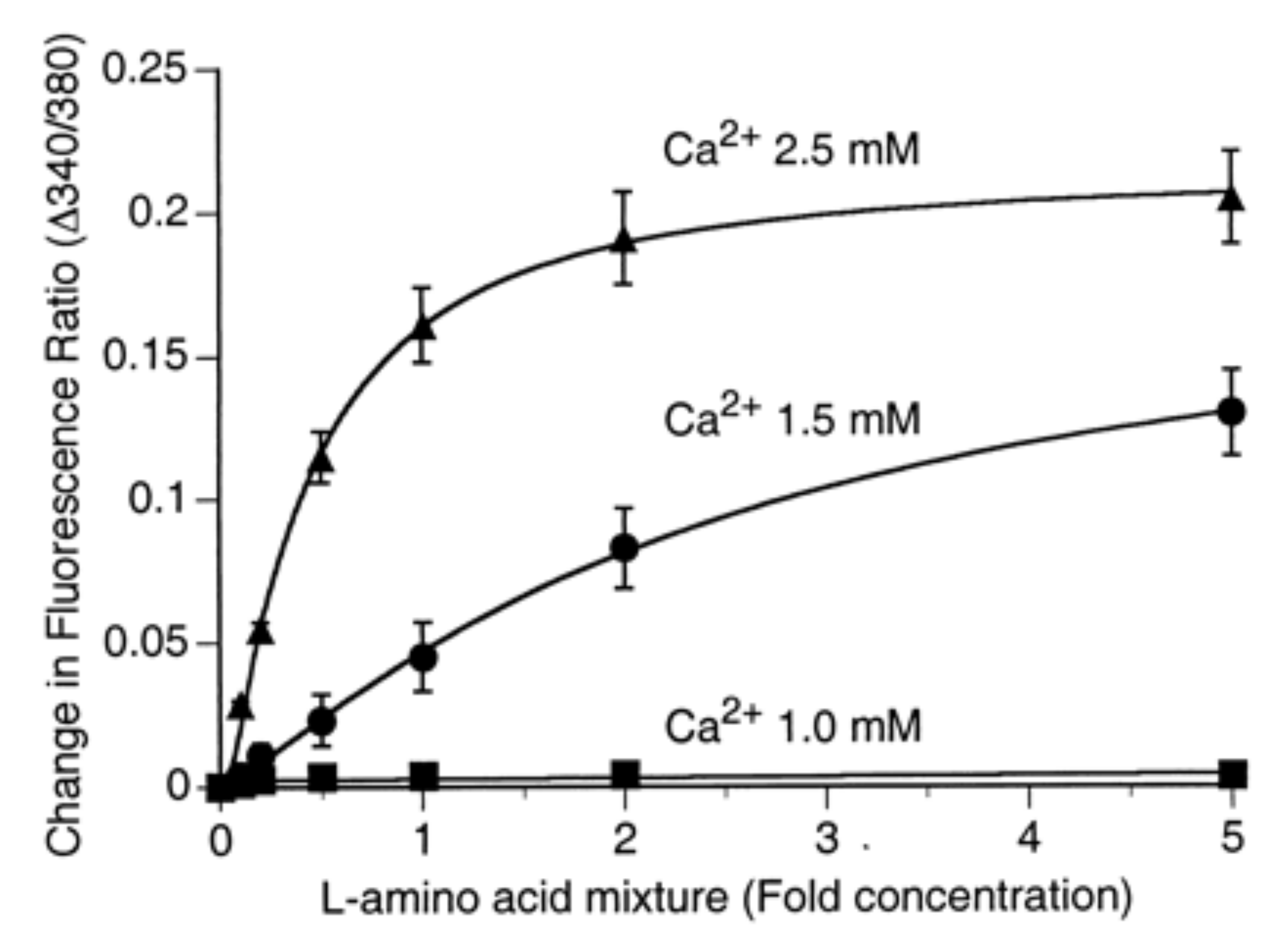
Differentiated tumor

Undifferentiated tumor



Chakhabaty et al., Cancer Res, 2003 and 2005

CaSR as a taste receptor for both Ca^{2+} and L-amino acids: L-amino acid mixtures enhance CaSR sensitivity for Ca^{2+}



Cells exposed to a mixture emulating the fasting plasma levels of the 20 common L-amino acids.

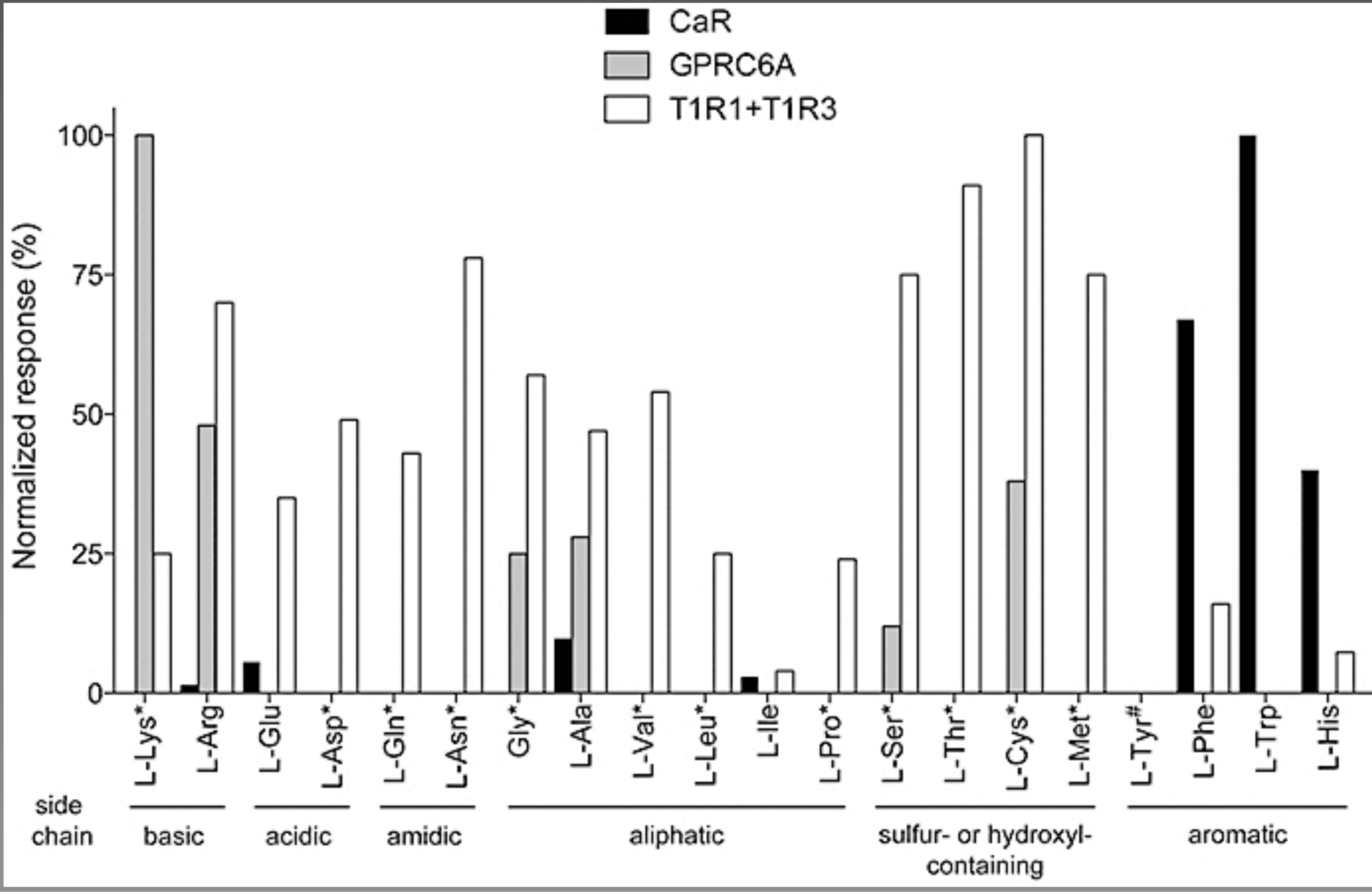
Conigrave et al (2000) PNAS

CaSR as a taste receptor for both Ca^{2+} and L-amino acids

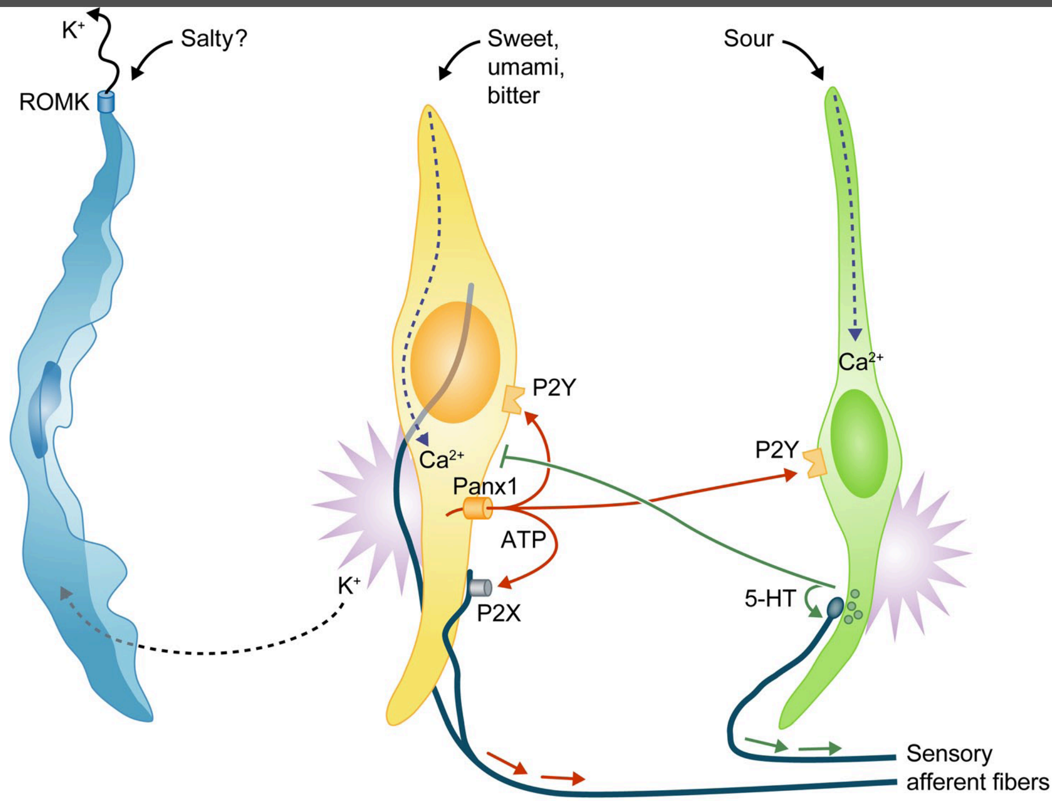
AA (10 mM)	EC50 for Ca^{2+}	ΔEC50 for Ca^{2+}	
Control	4.2	0	
L-His	2.4	1.9	basic
L-Phe	2.5	1.8	neutral-aromatic
L-Tyr	2.5	1.8	neutral-aromatic
L-Trp	2.6	1.6	neutral-aromatic
L-Cys	2.8	1.6	neutral-SH
L-Ala	2.9	1.4	neutral
L-Thr	3.0	1.1	neutral-OH
L-Asn	3.1	1.1	acidic
L-Gln	3.2	1.0	acidic
L-Ser	3.3	1.0	neutral-OH
L-Glu	3.5	0.9	acidic
Gly	3.6	0.7	neutral
L-Pro	3.6	0.6	Imino acid
L-Val	3.6	0.6	neutral
L-Met	3.6	0.6	neutral-SH
L-Asp	3.7	0.6	acidic
L-Lys	3.7	0.6	basic
L-Arg	3.7	0.5	basic
L-Ile	3.9	0.3	neutral
L-Leu	4.2	0	neutral

Conigrave et al (2000) PNAS

L-Amino acid selectivity profiles at CaSR, GPRC6A and the T1R1+T1R3 heterodimer (Wellendorph and Brauner-Osborne, 2009)



CaSR expression in taste cells



Type I glial-like cell

Neurotransmitter clearance	
GLAST	Glutamate reuptake
NTPDase2	Ecto-ATPase
NET	Norepinephrine uptake
Ion redistribution and transport	
ROMK	K^+ homeostasis
Other	
OXTR	Oxytocin signaling?

Type II receptor cell

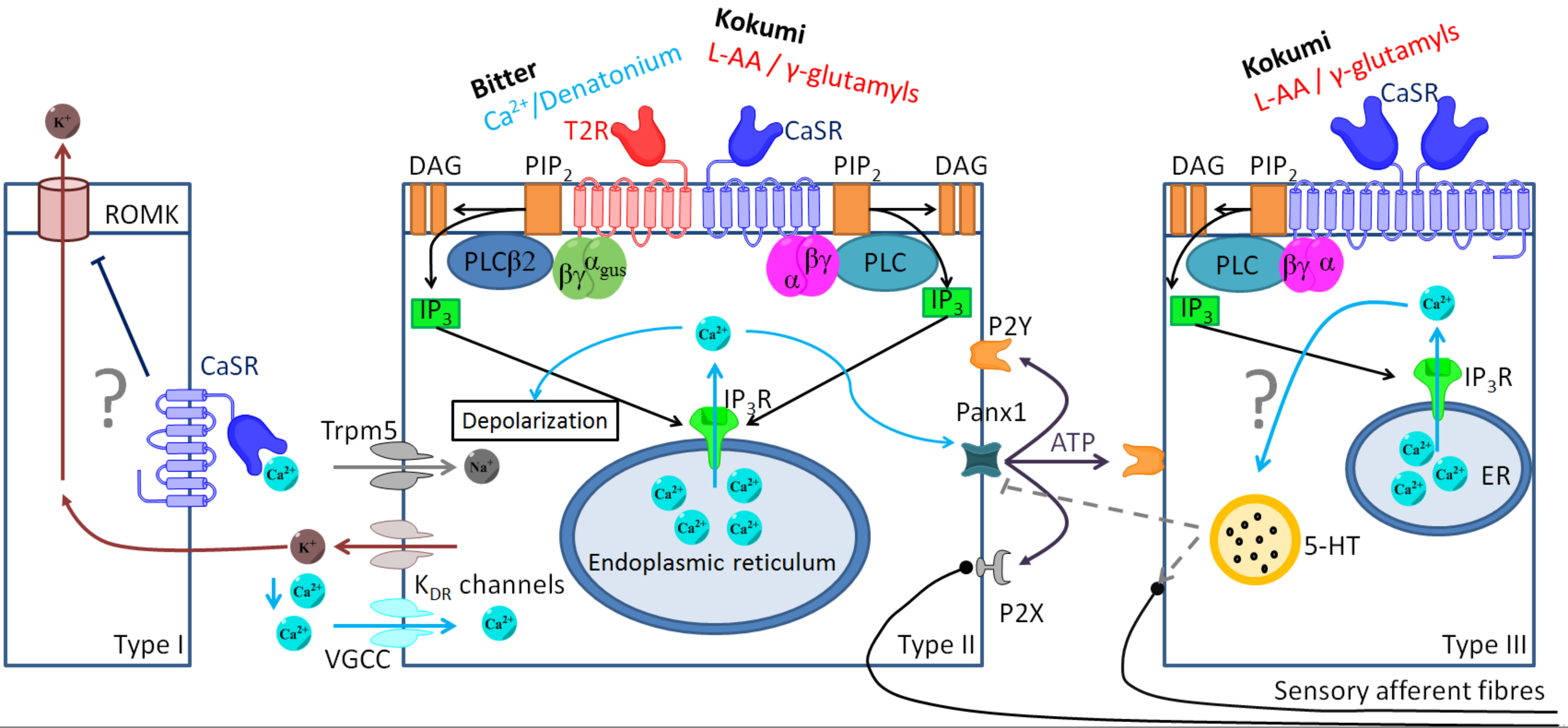
Taste transduction	
T1Rs, T2Rs	Taste GPCRs
mGluRs	Taste GPCRs
$G\alpha$-gus, $G\gamma13$	G protein subunits
PLCβ2	Synthesis of IP3
TRPM5	Depolarizing cation current
Excitation and transmitter release	
$Na_v1.7$, $Na_v1.3$	Action potential generation
Panx1	ATP release channel

Type III presynaptic cell

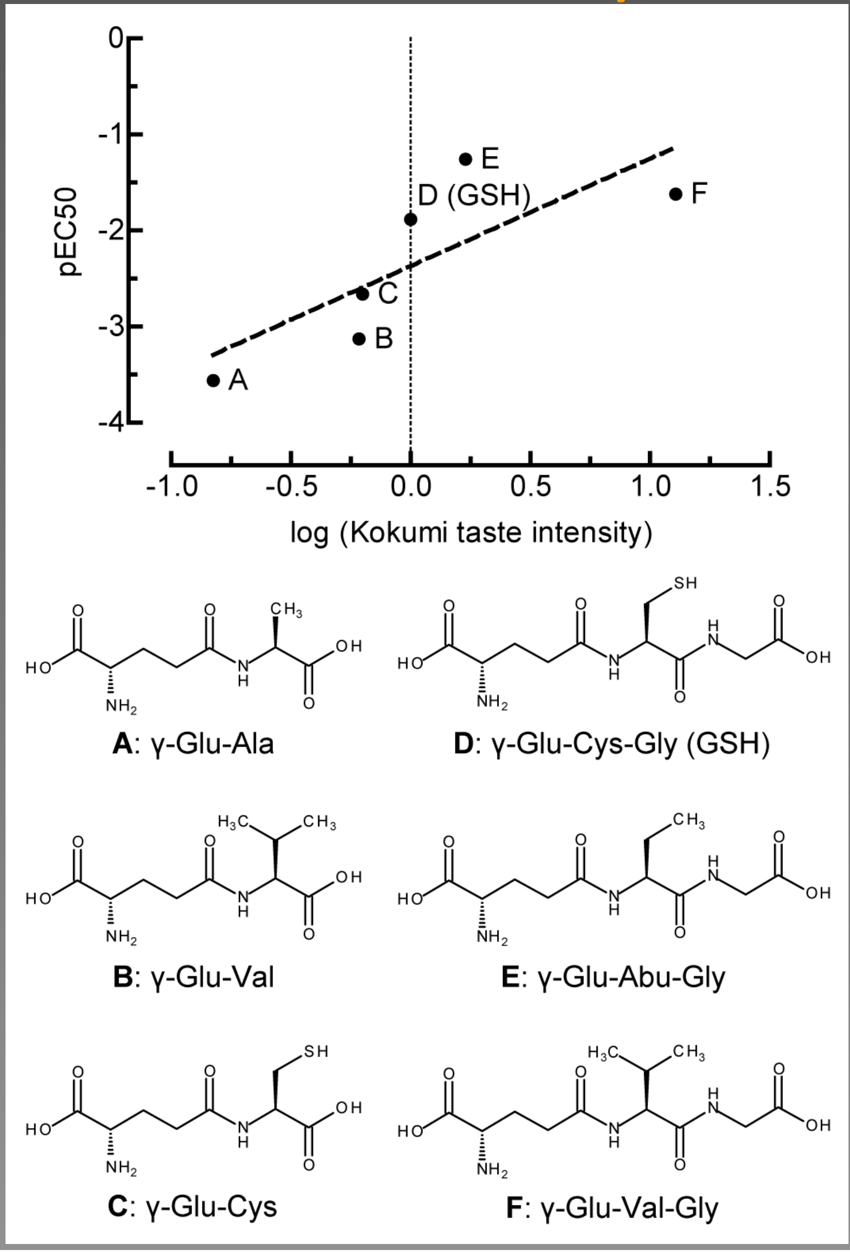
Surface glycoproteins, ion channels	
NCAM	Neuronal adhesion
PKD channels	Sour taste?
Neurotransmitter synthesis	
AADC	Biogenic amine synthesis
GAD67	GABA synthesis
5-HT	Neurotransmitter
Chromogranin	Vesicle packaging
Excitation, transmitter release	
$Na_v1.2$	Action potential generation
$Ca_v2.1$, $Ca_v1.2$	Voltage-gated Ca^{2+} current
SNAP25	SNARE protein, exocytosis

Chaudhari & Roper, 2010

Putative role(s) of the CaSR in Taste Cells

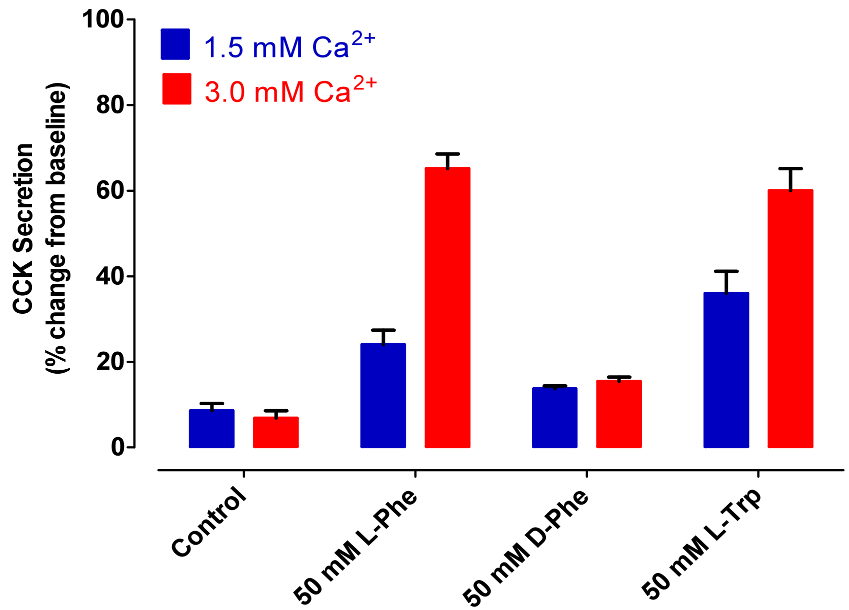


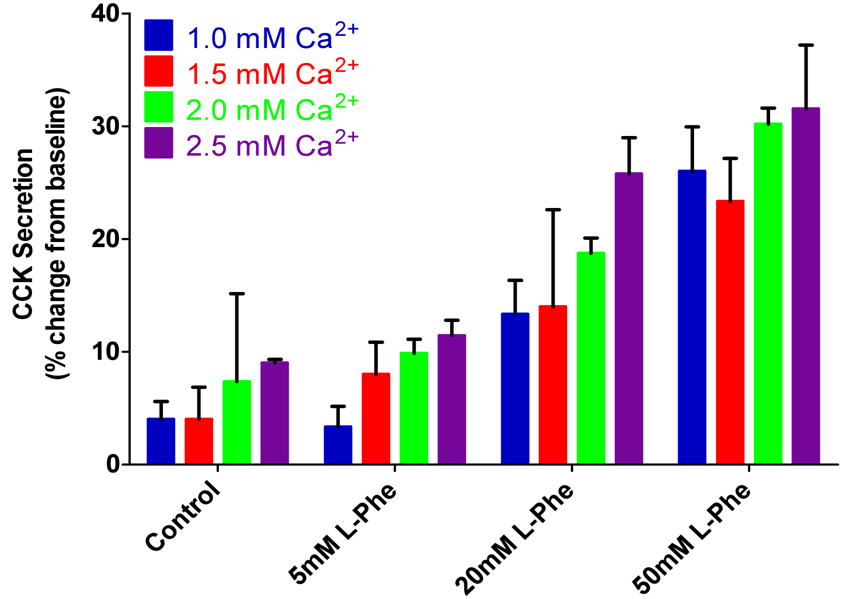
GSH and γ-glutamyl peptides act as "kokumi" taste substances (i.e, they enhance sweet, salty and umami tastes without producing a taste of their own)



Ohsu et al, 2010

CaSR activation evokes CCK secretion in STC-1 cells

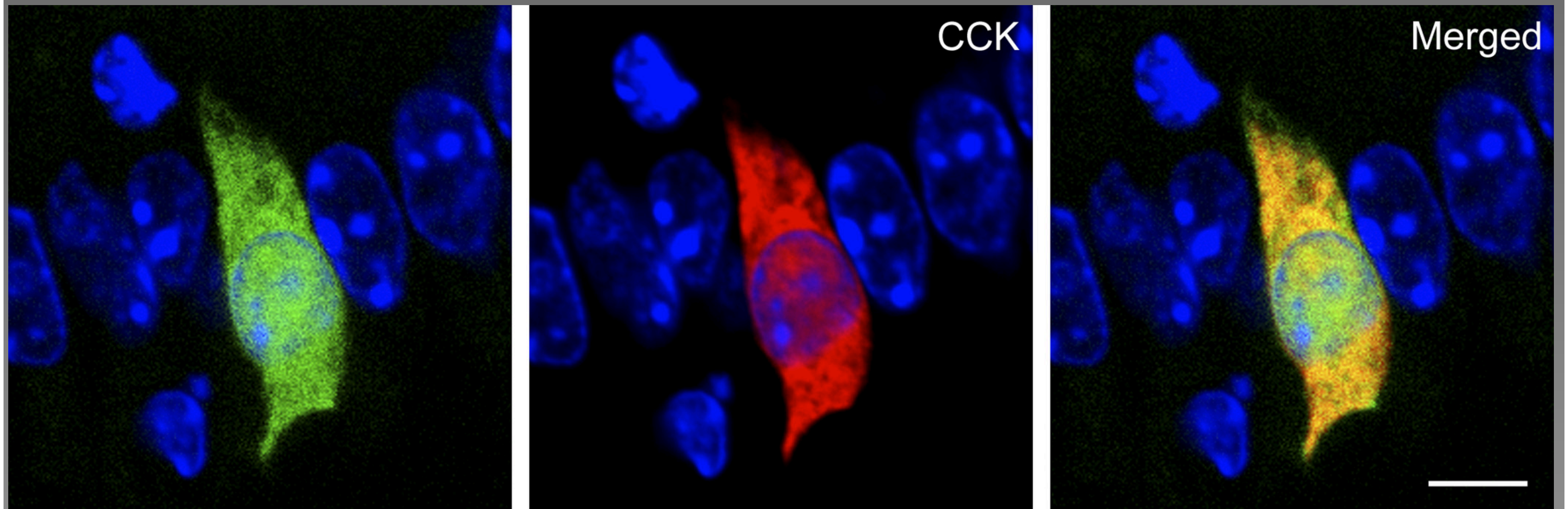




Brennan and Conigrave, unpublished

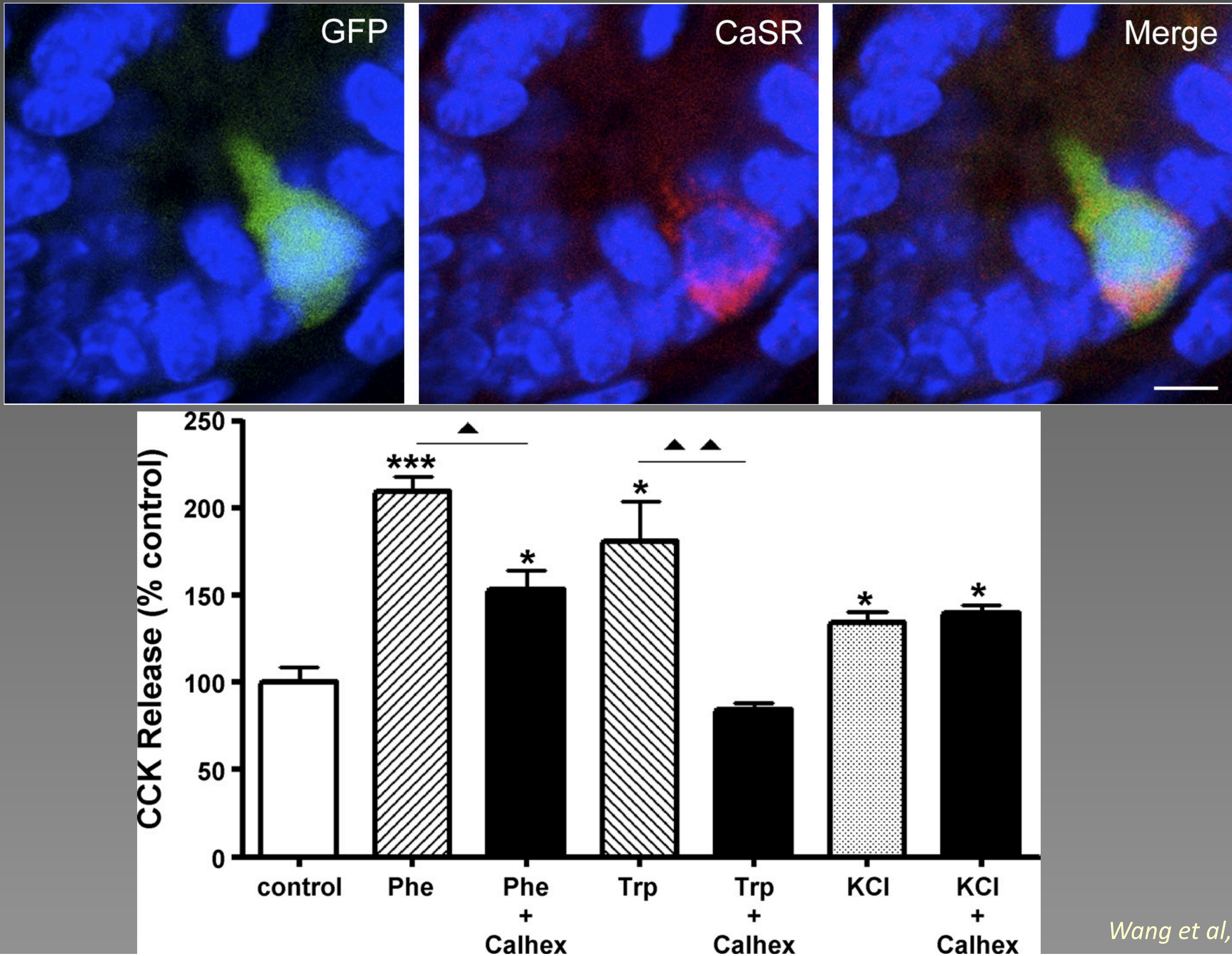
CaSR and regulation of CCK secretion

Wang et al, 2010 (CCK-secreting intestinal mucosal cells) used BAC transgenic mice for the identification of CCK-secreting cells by FACS sorting



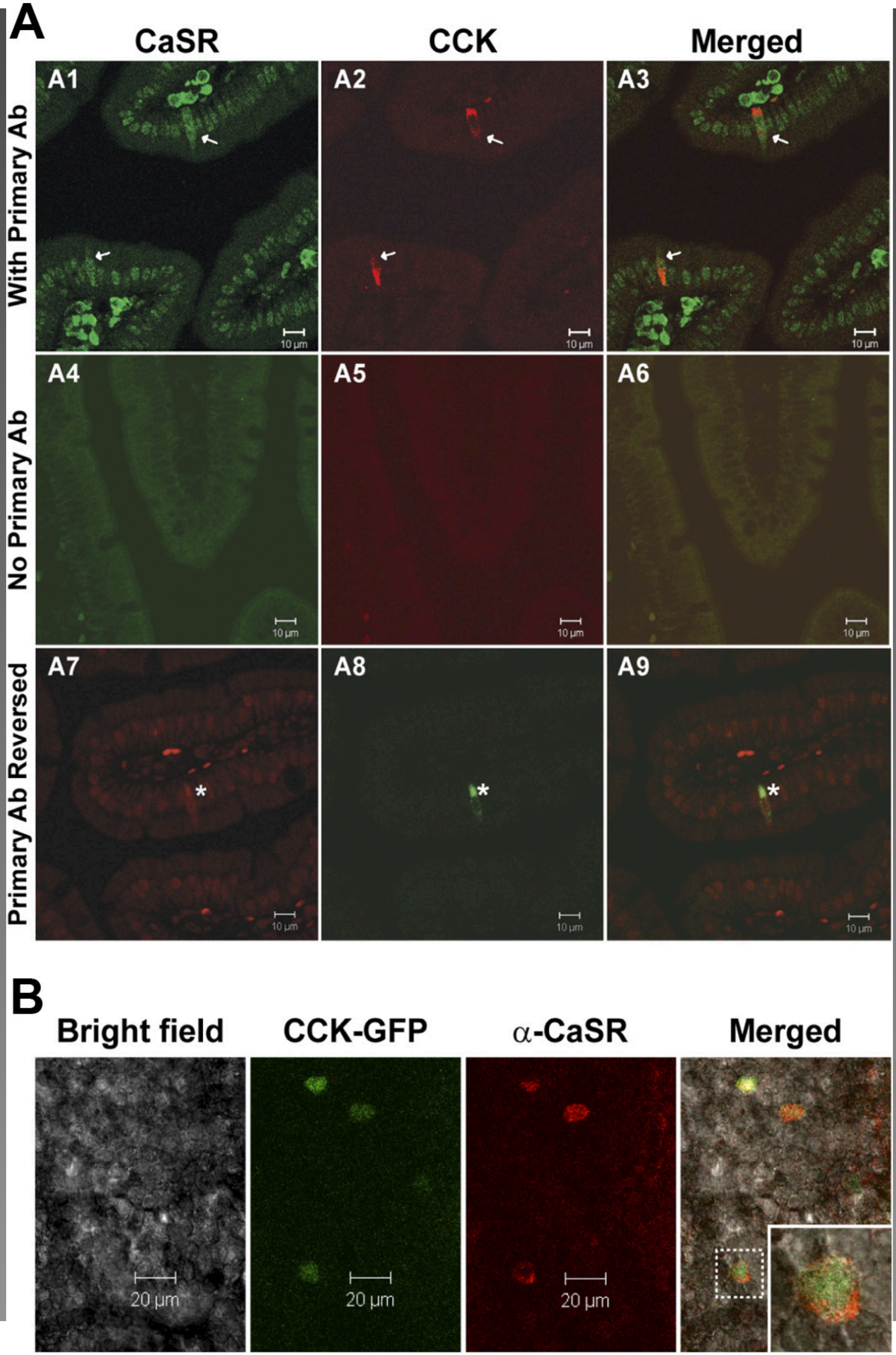
Wang et al, 2010

Functional expression of the CaSR in CCK-secreting cells



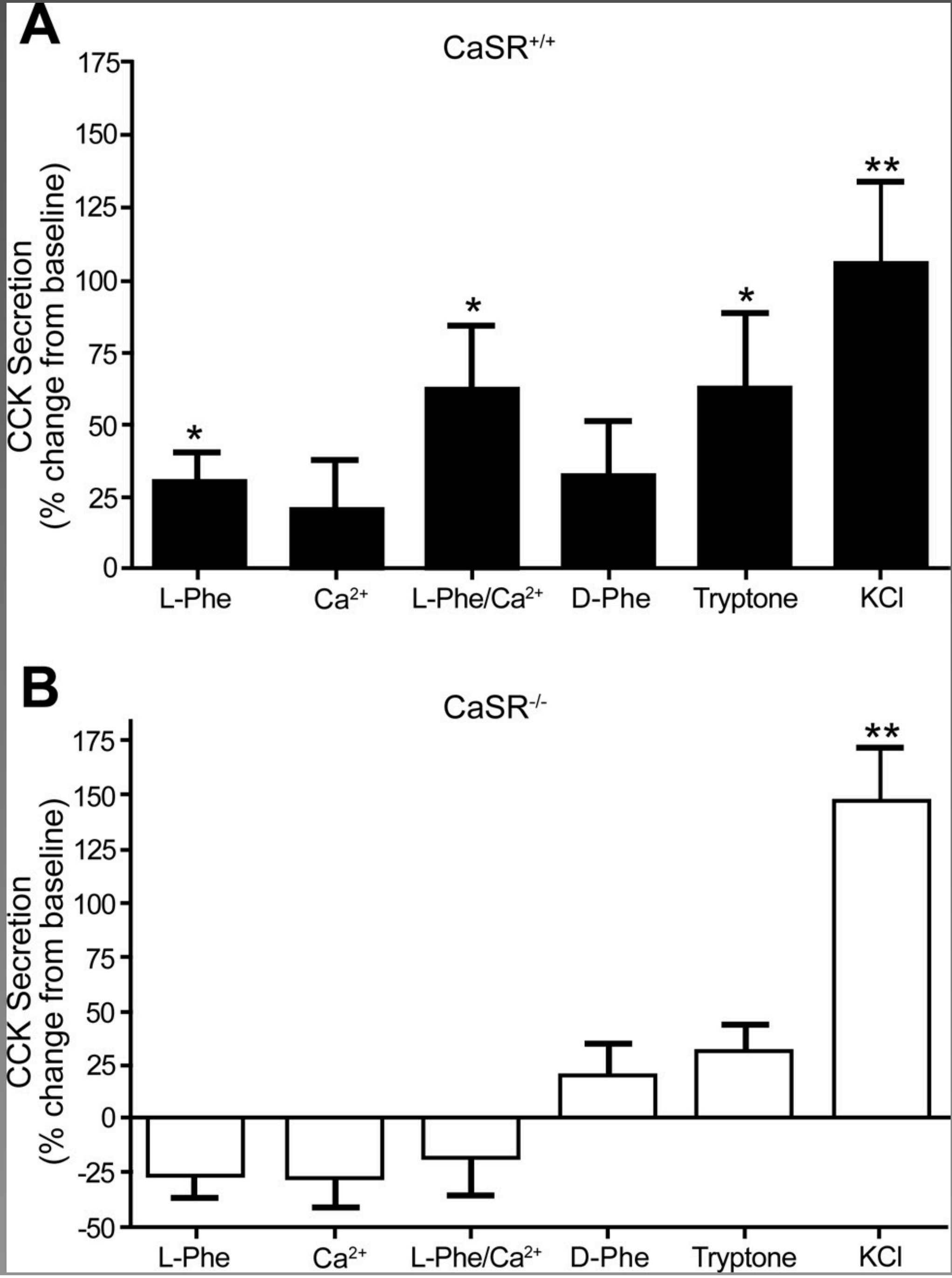
Wang et al, 2010

Liou et al, 2011 bred CaSR KO with eGFP CCK mice to isolate I cells



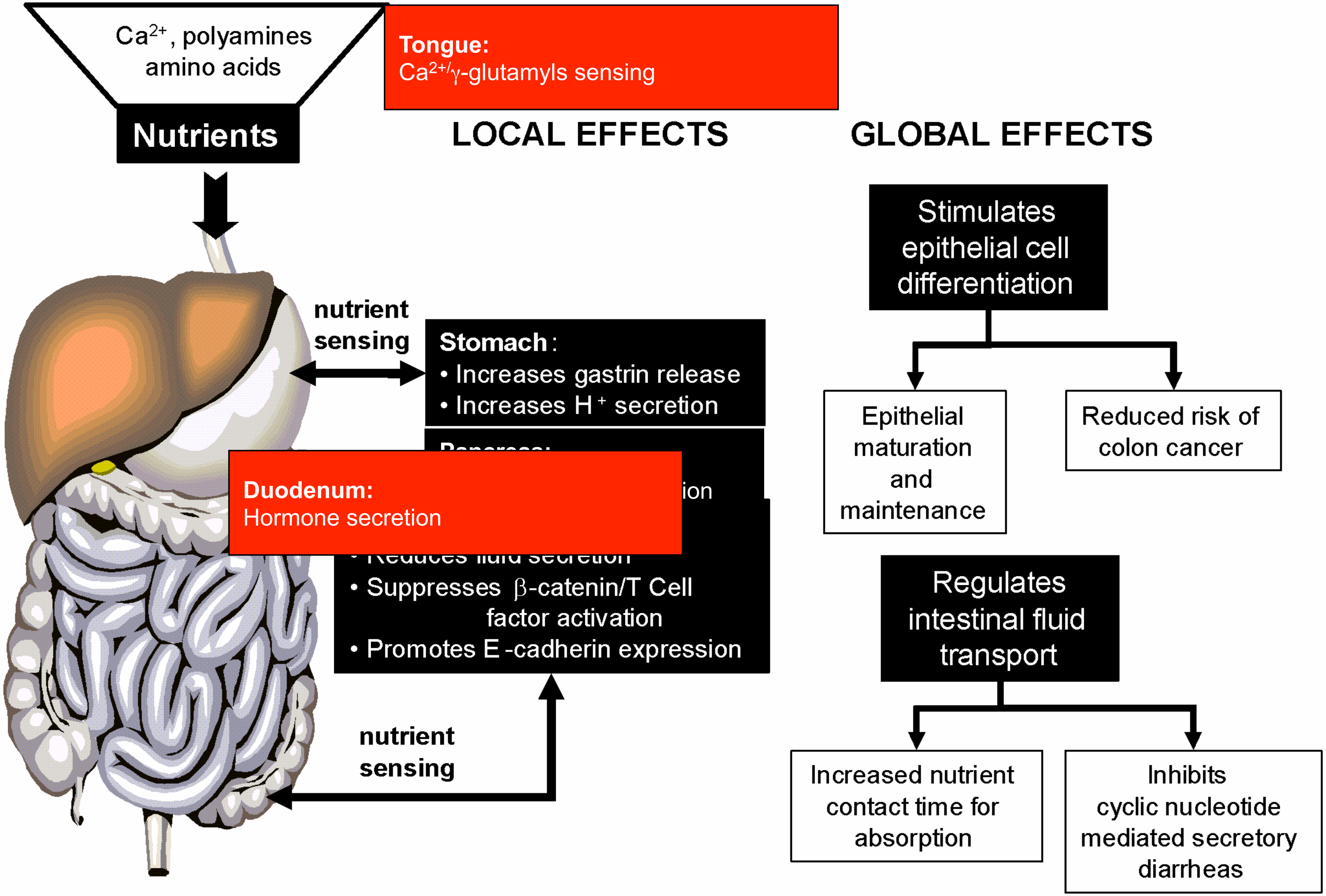
Liou et al, 2011

The CaSR as an amino acid sensor in I cells



Liou et al, 2011

CaR in the GI tract *(modified from Hebert et al., Cell Calcium 2004)*



Acknowledgements

S Brennan, P Yarova, T Davies, M Schepelmann, J Griffith, L Searchfield, R Wadey

Collaborators

- PJ Kemp, D Edwards, DFR Griffiths (Cardiff)
- D Mason, BAJ Evans (Cardiff)
- DT Ward, AE Canfield (Manchester)
- W Chang, D Bikle (UCSF)
- S Price (AZ, Macclesfield, UK)
- W Richards & L Ffrench (Amgen, USA and Amgen, UK)

Funding Bodies

- British Heart Foundation
- BBSRC-Amgen
- Astra Zeneca
- Arthritis Research UK Biomechanics and Bioengineering Centre
- Marie Curie ITN "Multifaceted CaSR"