PACAP & VIP

Prof. Debbie L. Hay

Department of Pharmacology & Toxicology, University of Otago, New Zealand

For further Q&A please email: debbie.hay@otago.ac.nz

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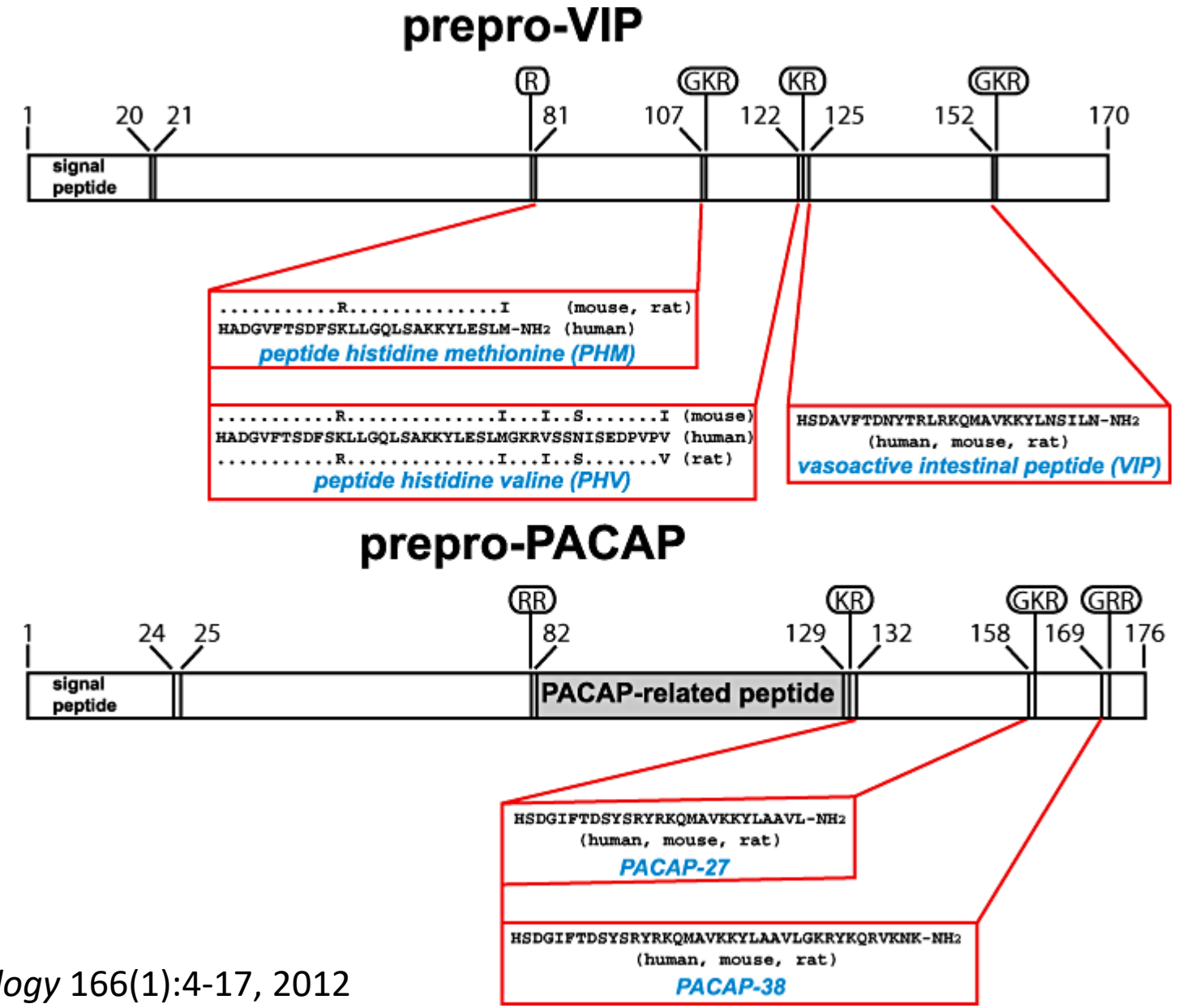
PACAP & VIP Peptide Family

- PACAP-38
- PACAP-27
- VIP
- PHM (PHI) and PHV

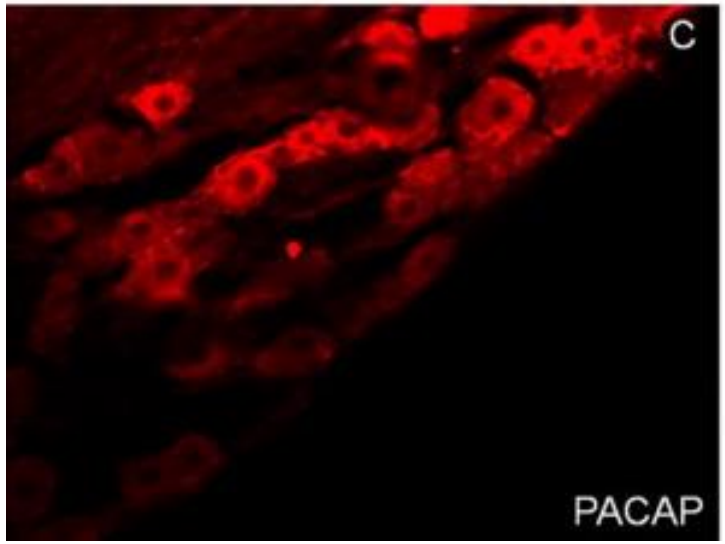


Harmar *et al.*, *British Journal of Pharmacology* 166(1):4-17, 2012

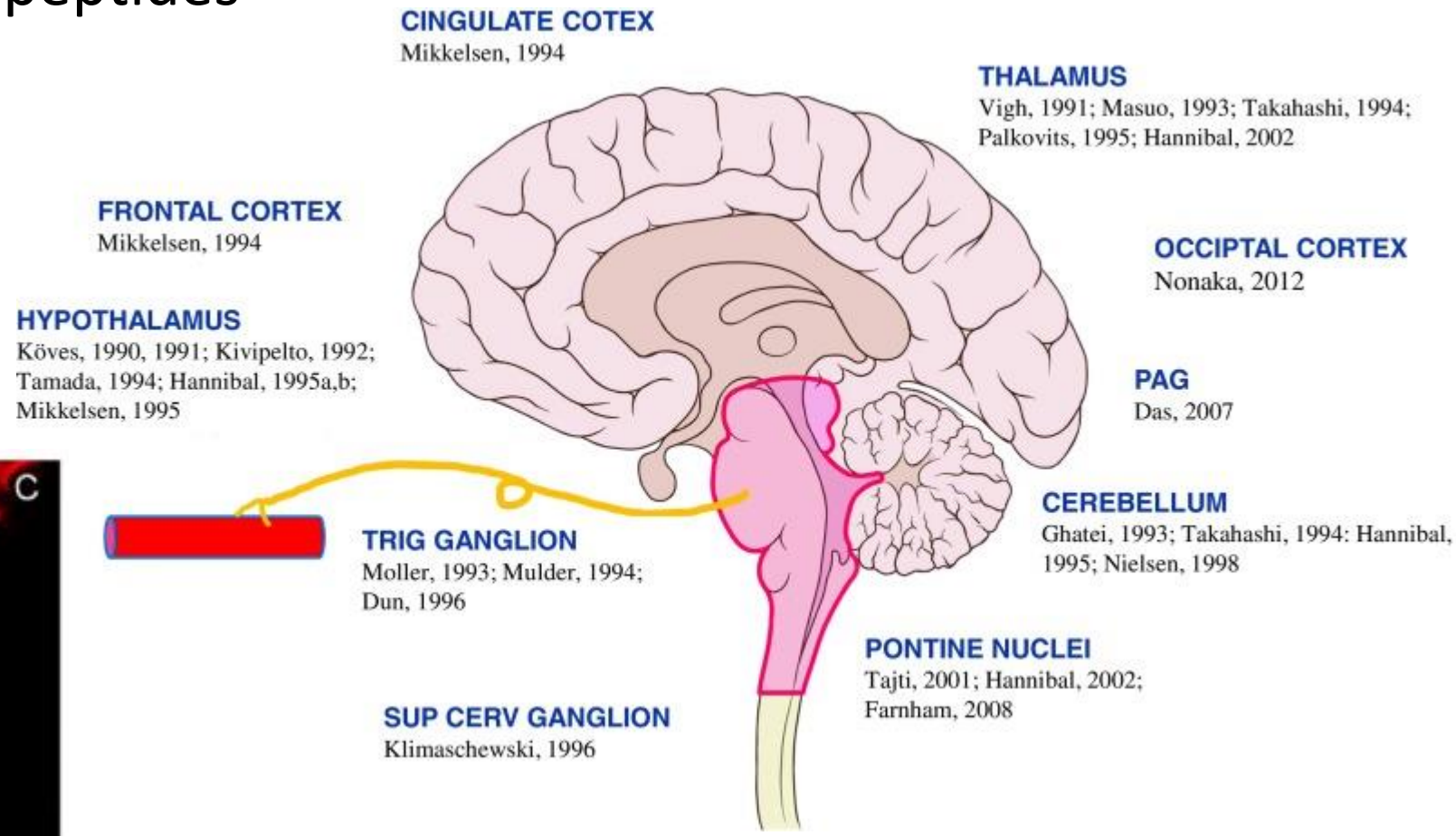
PACAP & VIP Peptide Family Expression

- Widely-expressed (neuro)peptides

e.g. PACAP



Jansen-Olesen *et al.*, *Neuropeptides*, 48(2):53-64, 2014



Vollesen *et al.*, *Neurotherapeutics,* 15(2): 371–376, 2018

Migraine – Clinical Evidence

- No change/increase/decrease in PACAP-like immunoreactivity reported in migraine

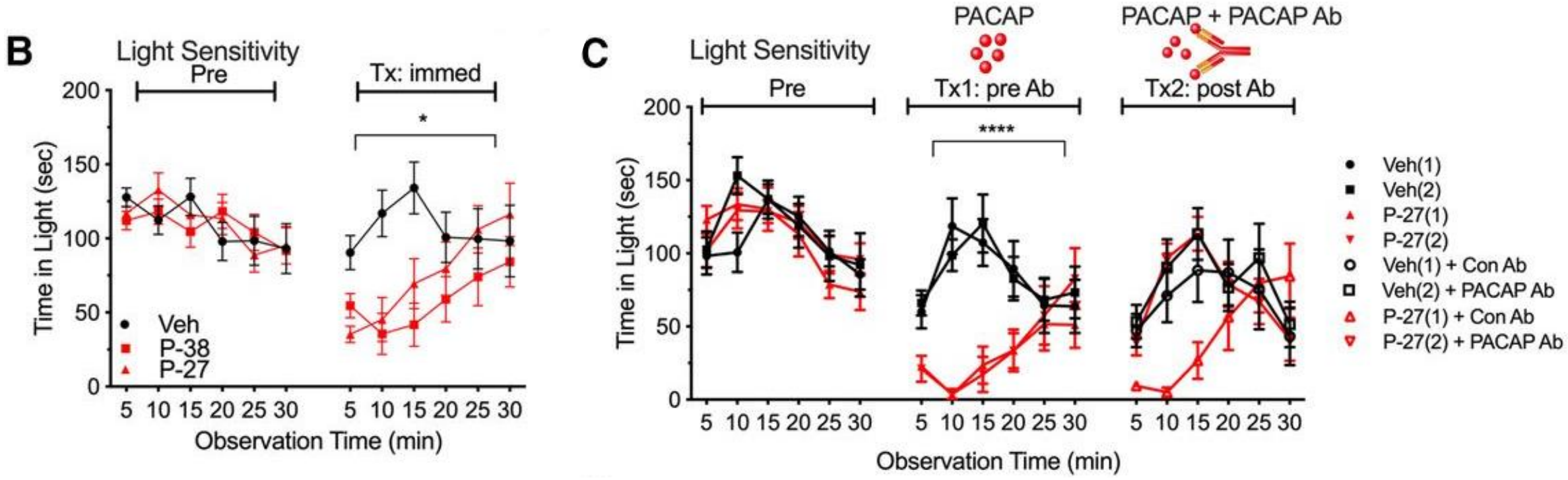
  e.g. Tuka *et al*., 2013; Zagami *et al*., 2014; Cernuda-Morollón *et al*., 2016

- Infusion of PACAP-27, PACAP-38 and prolonged (2 hour) VIP trigger migraine-like attacks in humans

  e.g. Schytz *et al.*, 2009*;* Amin *et al.,* 2014; Guo *et* al., 2017; Ghanizada *et al.,* 2020, Pellesi *et al*., 2021

Migraine - Pre-clinical Evidence

- Vasodilation, neuronal sensitization, mast-cell degranulation
  e.g. Ackerman *et al*., 2015*;* Baun et *al.,* 2012*;* Bhatt *et al*., 2014; Guo *et al*., 2021
- PACAP-induced photophobia in mice



Kuburas *et al*., *J Neurosci.,* 41(21): 4697-4715, 2021

A Distinct Pathway From CGRP?

- PACAP-induced photophobia not attenuated by CGRP blockade.
- CGRP-induced photophobia not attenuated by PACAP blockade.

  Kuburas *et al., J. Neurosci.,* 2021
- PACAP and CGRP co-expressed and found in separate neurons in trigeminal ganglia

  Jansen-Olesen *et al*., *Neuropeptides*, 2014; Eftekhari *et al.*, *Brain Research,* 2015
- Separate populations of PACAP and CGRP-responsive neurons in trigeminal ganglia.

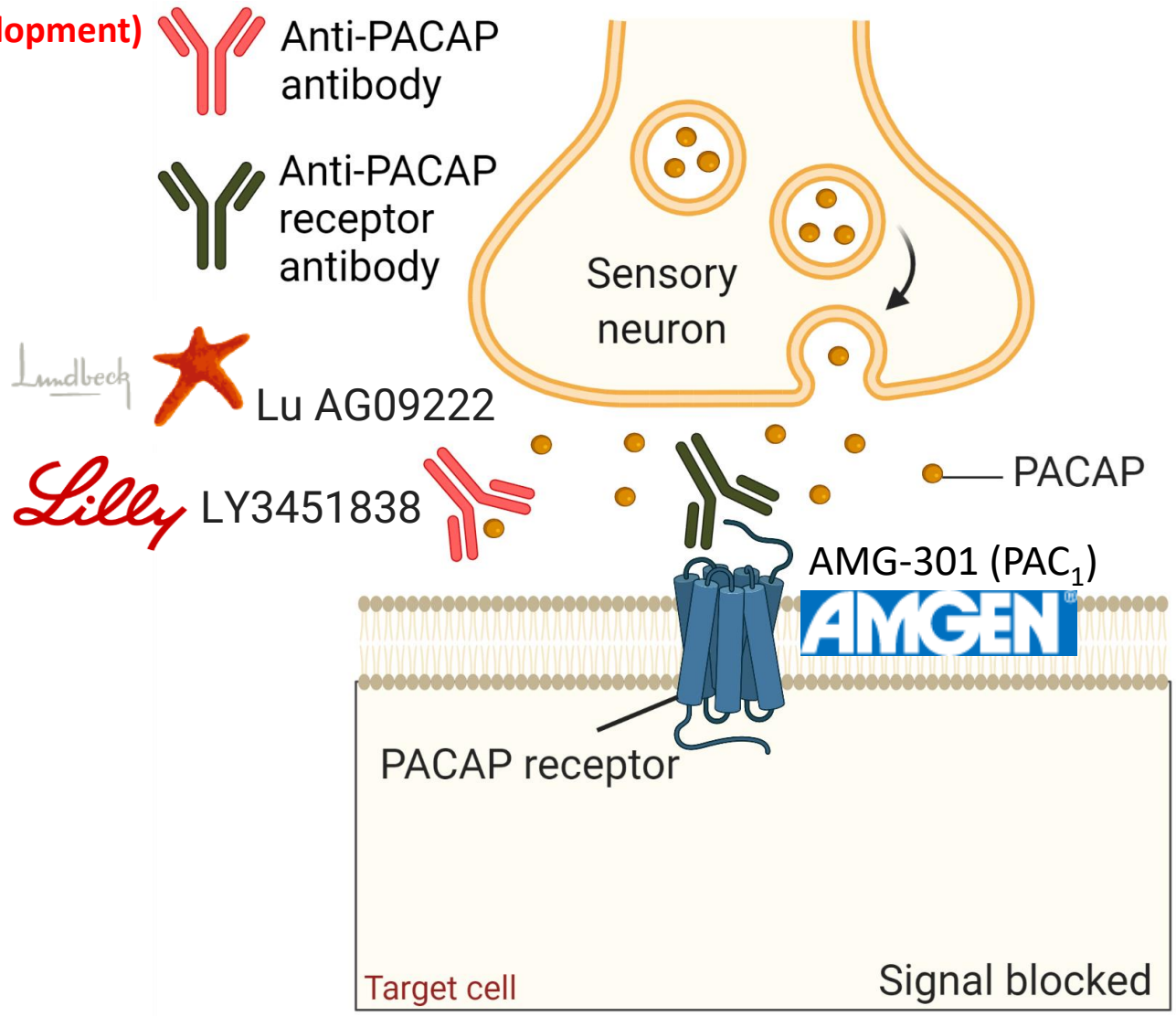
  Guo *et al., Pain*, 2021
- PACAP-38 (not -27 or VIP) causes CGRP release from the trigeminal nucleus caudalis but not the trigeminal ganglia

  Jansen-Olesen *et al*., *Neuropeptides*, 2014

Approaches To Targeting VIP/PACAP In Migraine (non-approved drugs under development)

- Strategies mirror CGRP
- Target peptides with antibodies
- Target receptors with antibody antagonists
- Target receptors with small molecule or peptide antagonists
- Current efforts have been directed towards PACAP – peptide and receptor mAbs
- No benefit from AMG-301 for migraine prevention

  Ashina *et al.*, *Cephalalgia*, 2021



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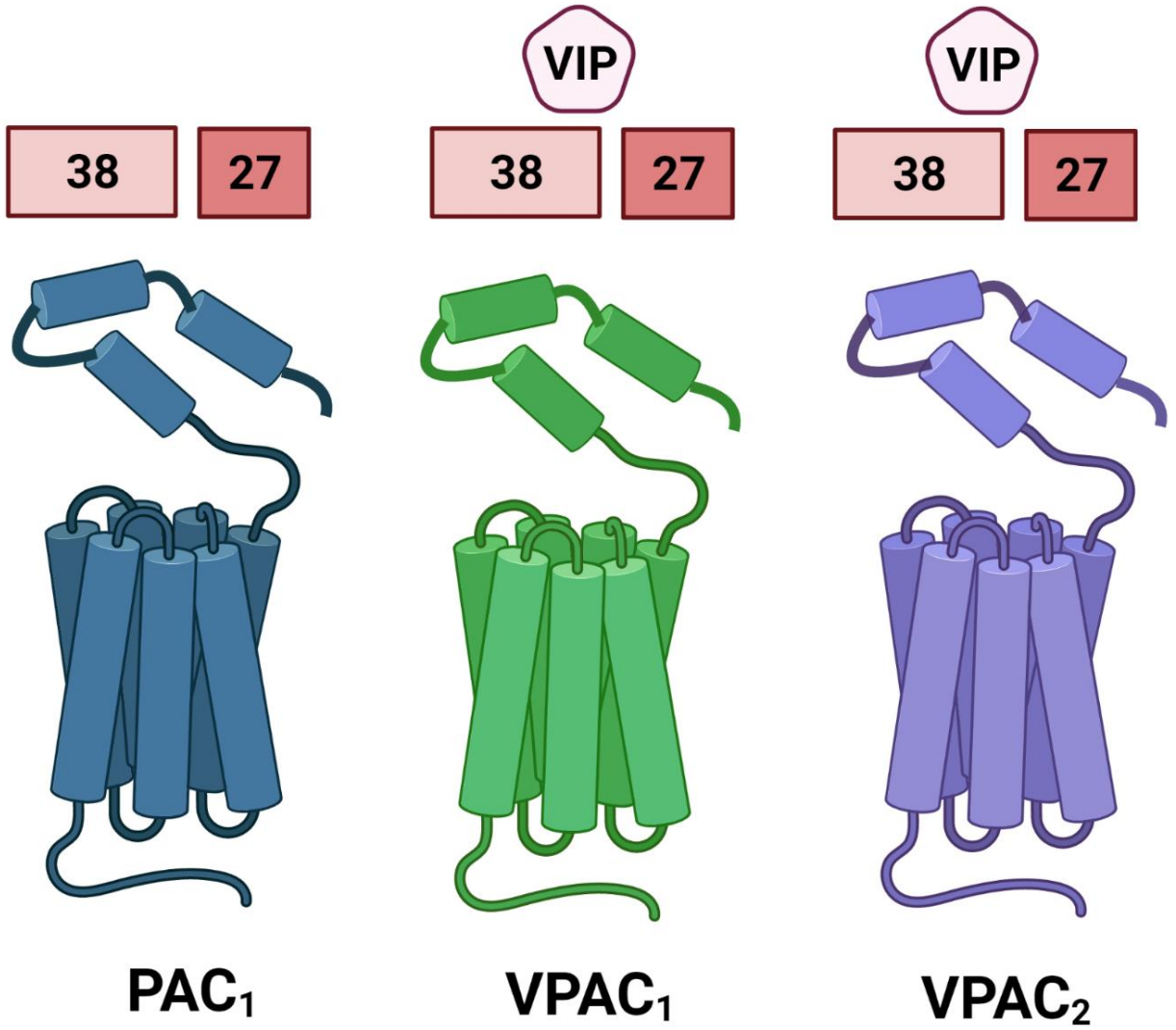
Therapeutic Strategy?

- Which peptide(s) is most important?
- Which location(s) (central vs. peripheral?)
- Which receptor(s) is most important?

VIP & PACAP Receptors

- Three G protein-coupled receptor genes – *ADCYAP1R1*, *VIPR1*, *VIPR2*, encoding PAC_1, $VPAC_2$ and $VPAC_2$ receptors, respectively
- Figure shows a simplified view of their relative affinities for VIP and PACAP
- Actual situation is much more complex



https://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=67

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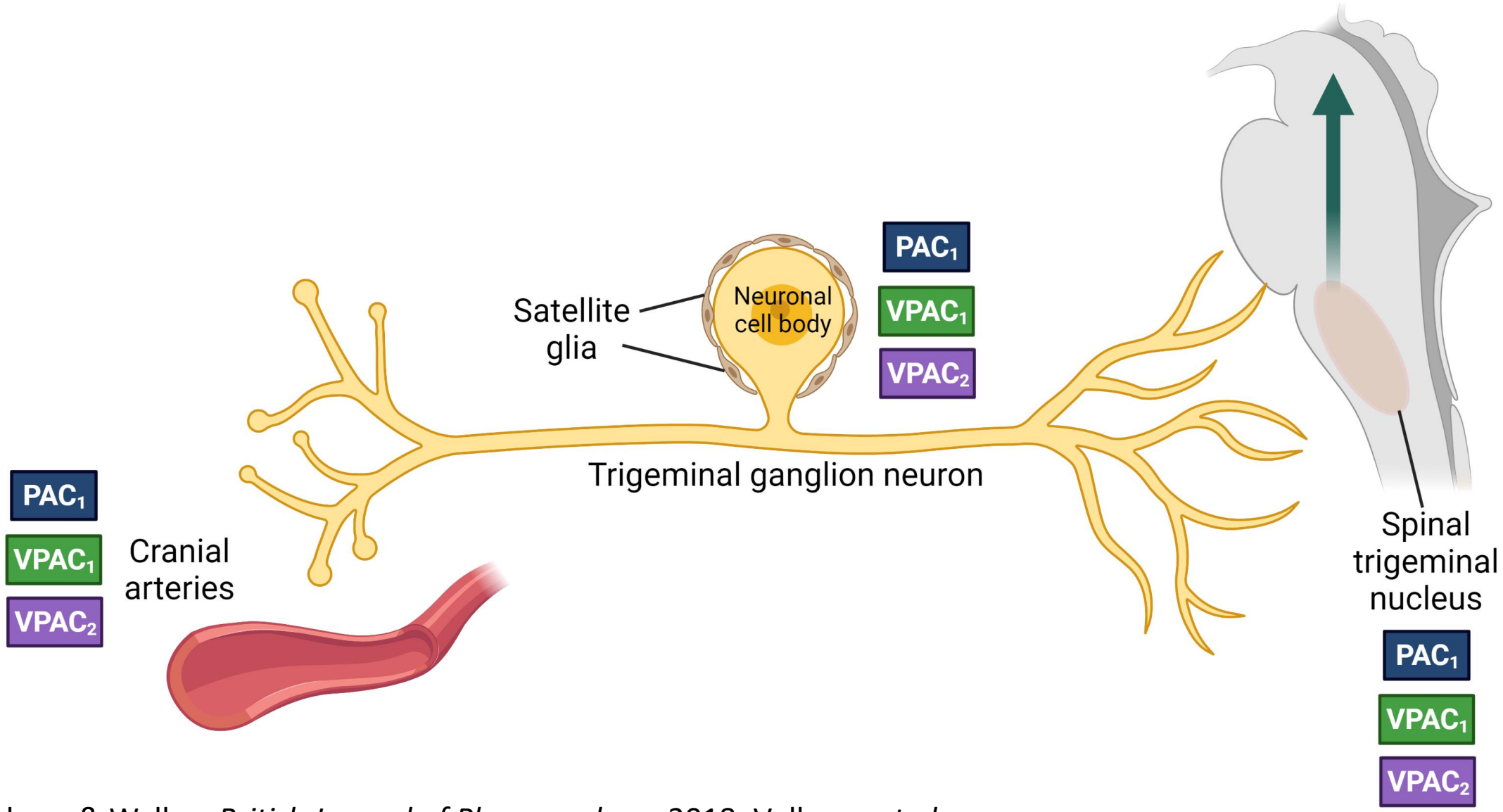
Receptor Expression



Sundrum & Walker, *British Journal of Pharmacology*, 2018; Vollesen *et al*., *Neurotherapeutics,* 2018; Jansen-Olesen *et al., Neuropeptides*, 2014

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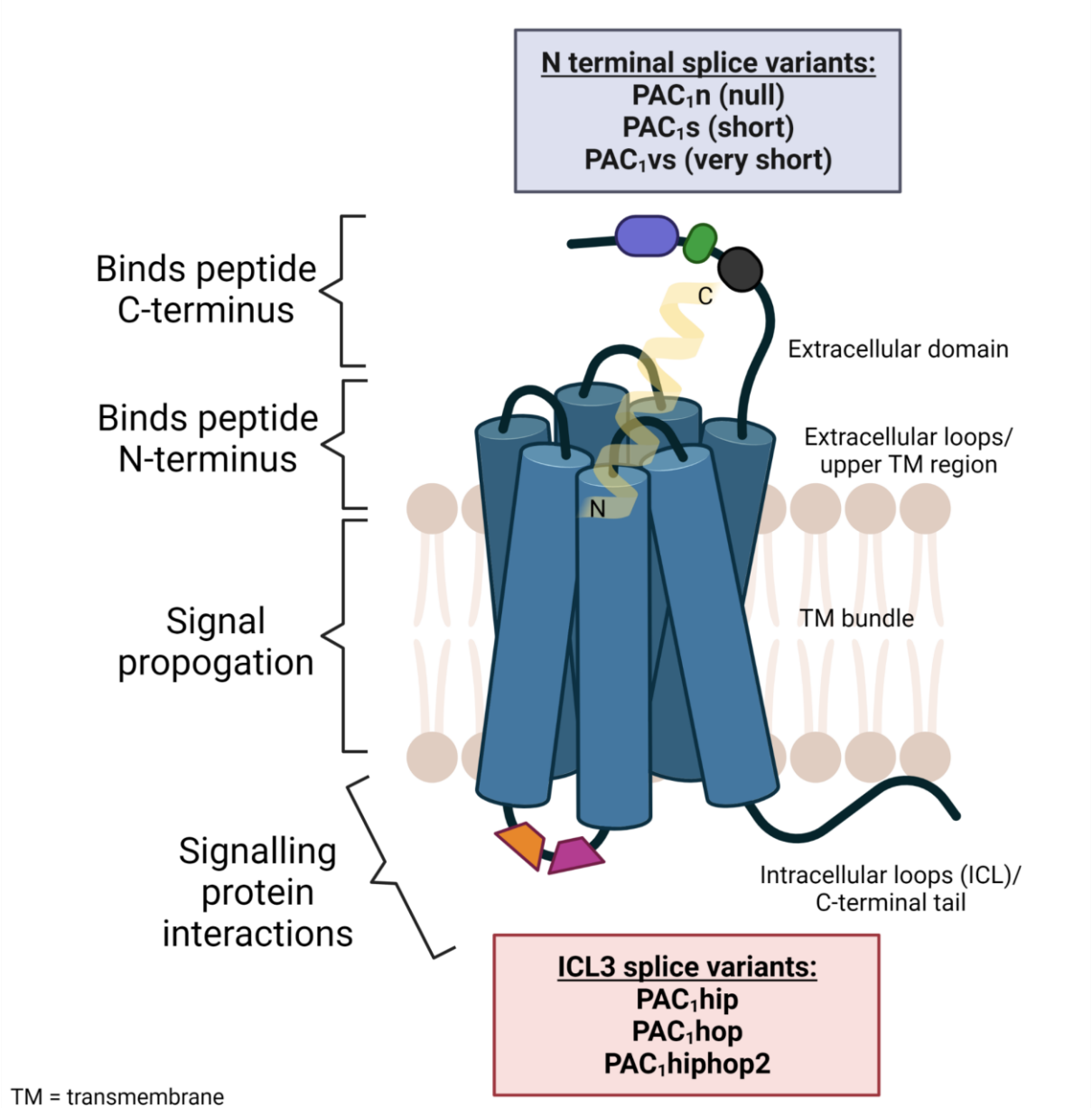
PAC_1 Receptor Splice Variants



- PAC_1 receptor has multiple exons
- Variable inclusion leads to multiple mature protein sequences
- Sites altered affect ligand binding and cell signalling
- Complexity creates challenges for understanding receptor function
- Limited information on $VPAC_1$ or $VPAC_2$ splicing – both contain multiple exons so this is possible

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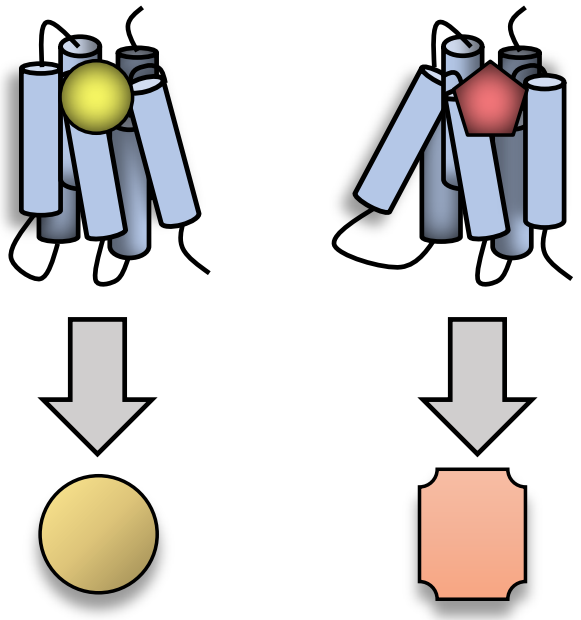
Other Factors

- Signalling bias



- GPCRs are flexible proteins – many possible conformations.
- Ligands of different shapes and sizes stabilize unique GPCR conformations.
- Leads to preferential coupling to different intracellular signaling proteins and different effects.

- Agonist and signalling pathway-dependent antagonism

Walker *et al*., *Cephalalgia,* 2018; Tasma *et al*., *Pharmacol. Res. Perspect.*, 2020

Receptor-Focused Questions

To effectively target this system we need to know:

- which receptor subtypes are involved,
- which signalling pathways are involved,
- whether all signalling pathways are capable of being blocked,
- where each receptor is located

Goal & Approach

Establish reference framework of receptor pharmacology and signalling in transfected cell systems (human receptors, Cos7)

<u>Agonist analysis</u>

- Four receptors (PAC_{1n}, PAC_{1s}, $VPAC_1$, $VPAC_2$)
- Five agonists (PACAP-27, PACAP-38, VIP, PHM, maxadilan)
- Five signalling molecules (cAMP, IP_1, pAkt, pERK, pCREB)

<u>Antagonist analysis (cAMP only)</u>

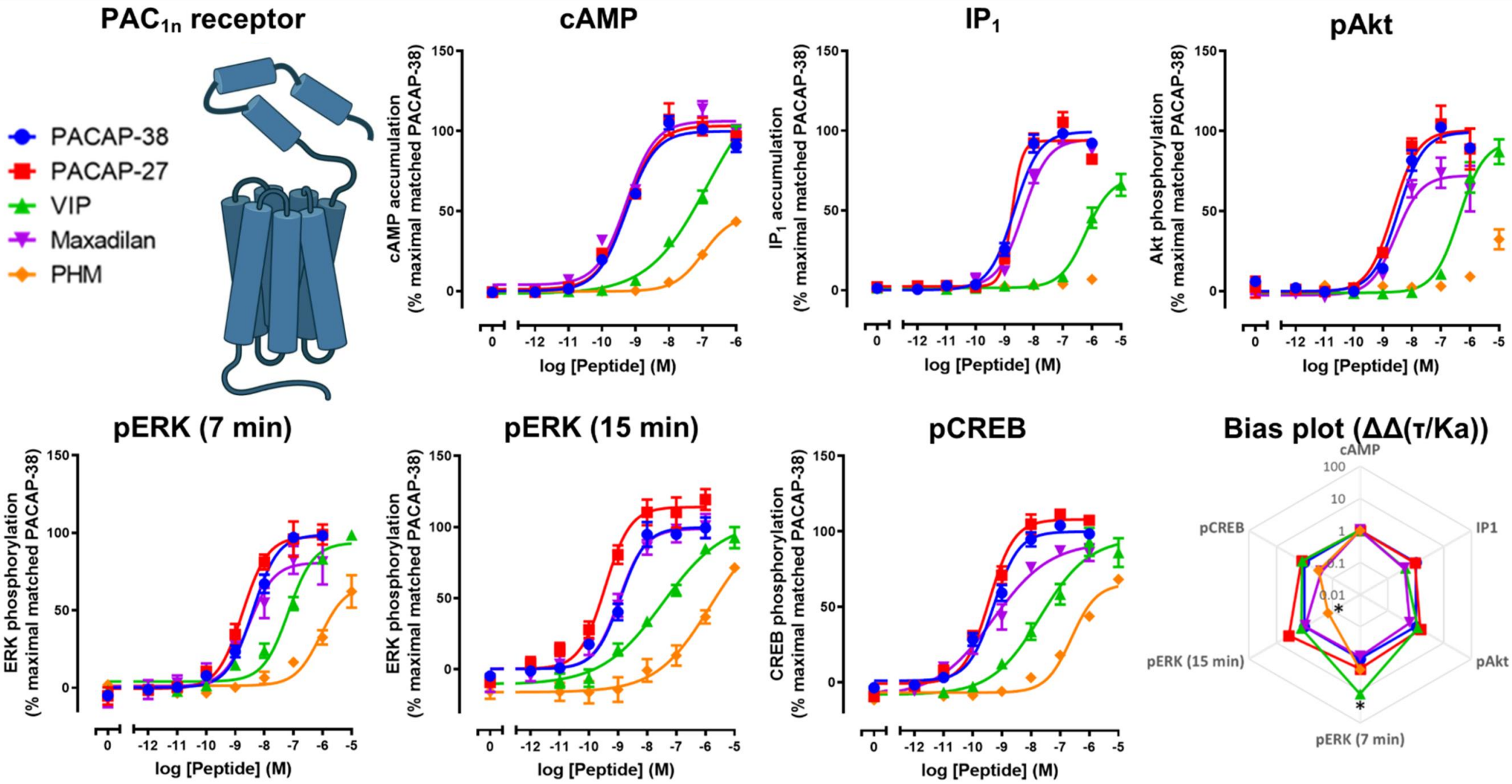
- Four receptors (PAC_{1n}, PAC_{1s}, $VPAC_1$, $VPAC_2$)
- Three agonists (PACAP-27, PACAP-38, VIP)
- Three antagonists (PACAP-38, PG 97-269, M65)

Presenting key results.

For full data see Tasma *et al*., *British Journal of Pharmacology,* in press 2021

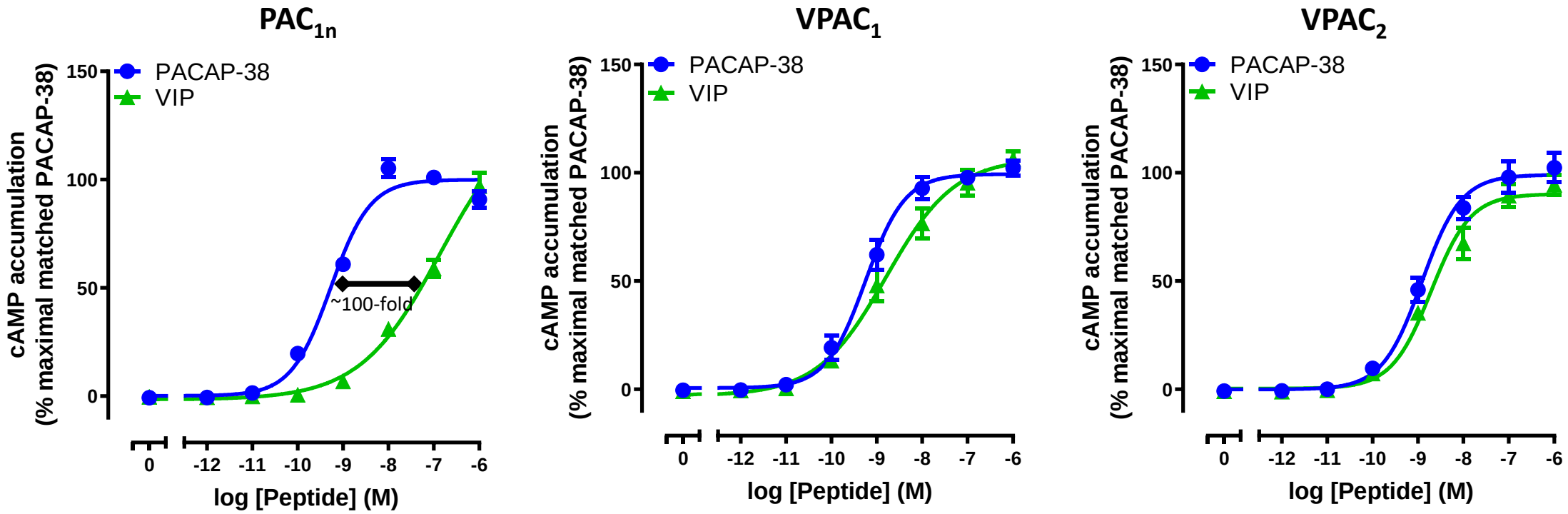
doi: 10.1111/bph.15700

Example Agonist Data:



PAC_{1n}, PAC_{1s}, $VPAC_1$, $VPAC_2$ receptors display similar patterns of signalling

PAC_{1n} vs. VPAC

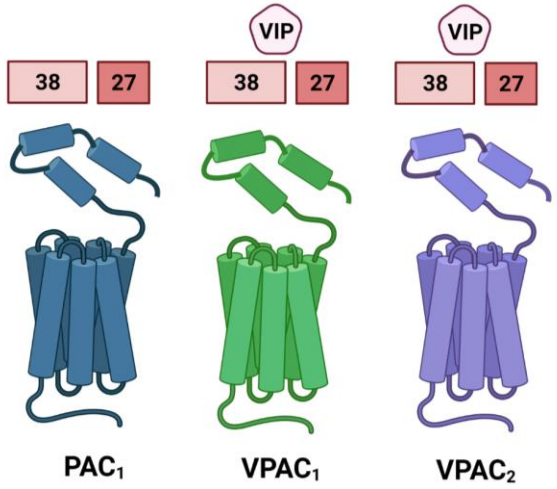


Relative potency of PACAP-38 and VIP consistent with overall view of receptor pharmacology:

PAC_{1n} PACAP-38>>VIP

$VPAC_1$ VIP=PACAP-38

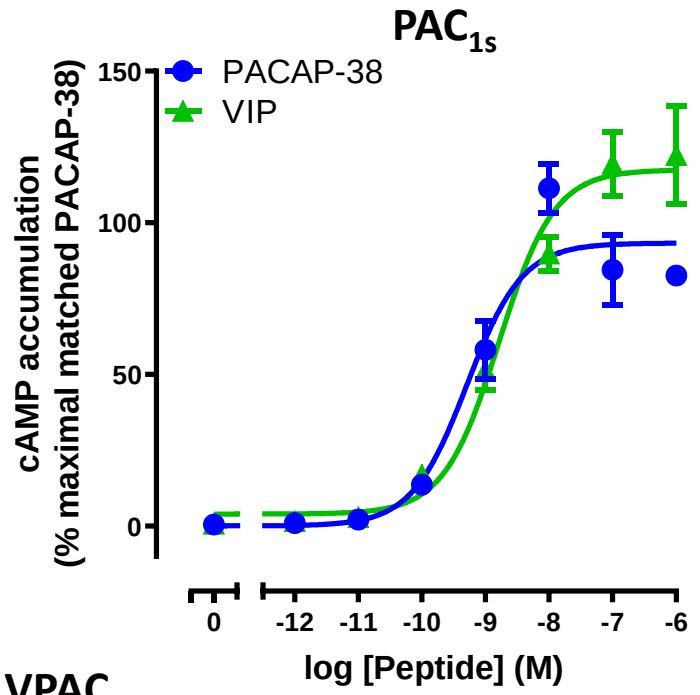
$VPAC_2$ VIP=PACAP-38

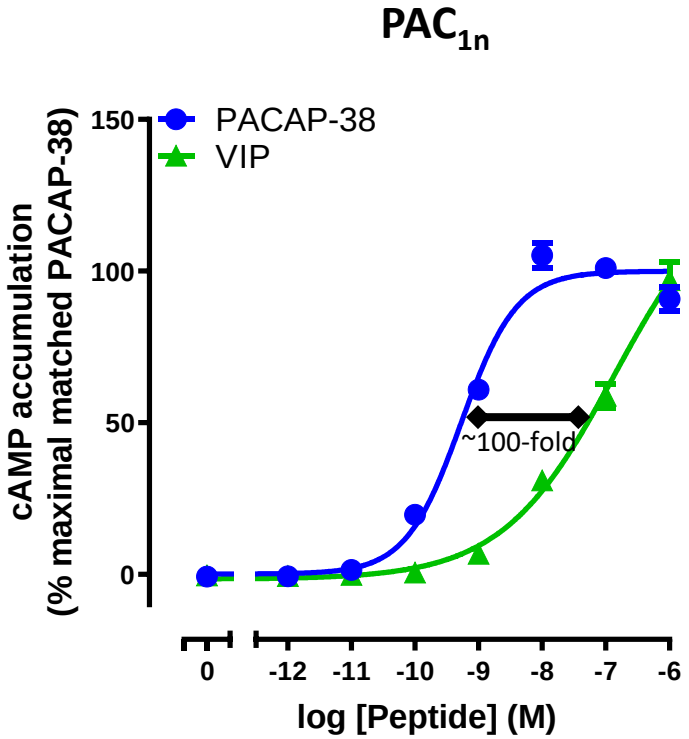


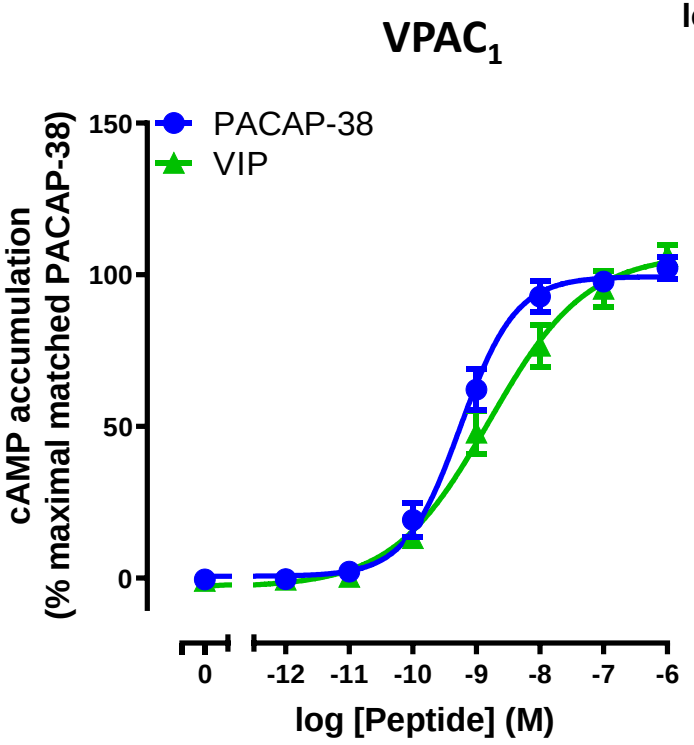
PAC_{1s} vs. VPAC

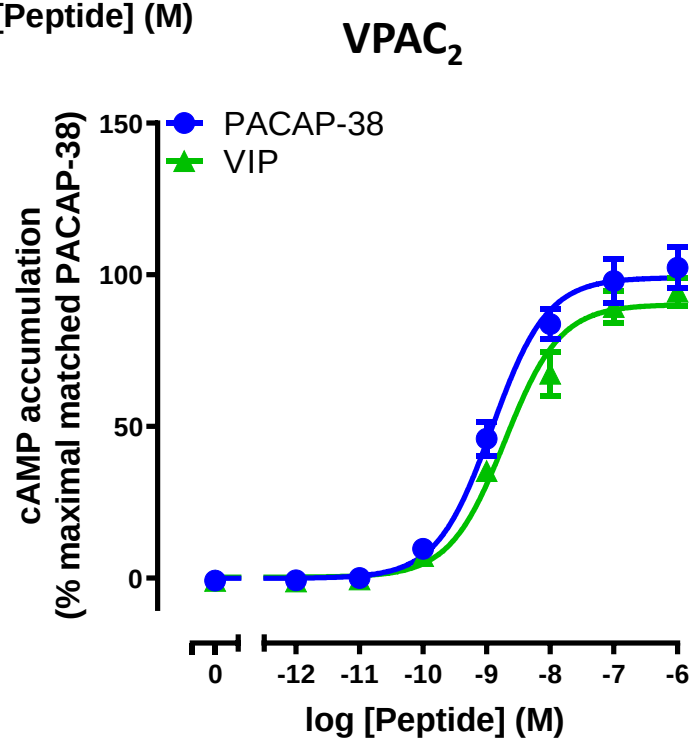
Relative potency of PACAP-38 and VIP <u>not</u> consistent with overall view of receptor pharmacology: PAC_{1s} PACAP-38=VIP

(Consistent with Dautzenberg et al., J. Neuroendocrinol, 1999)

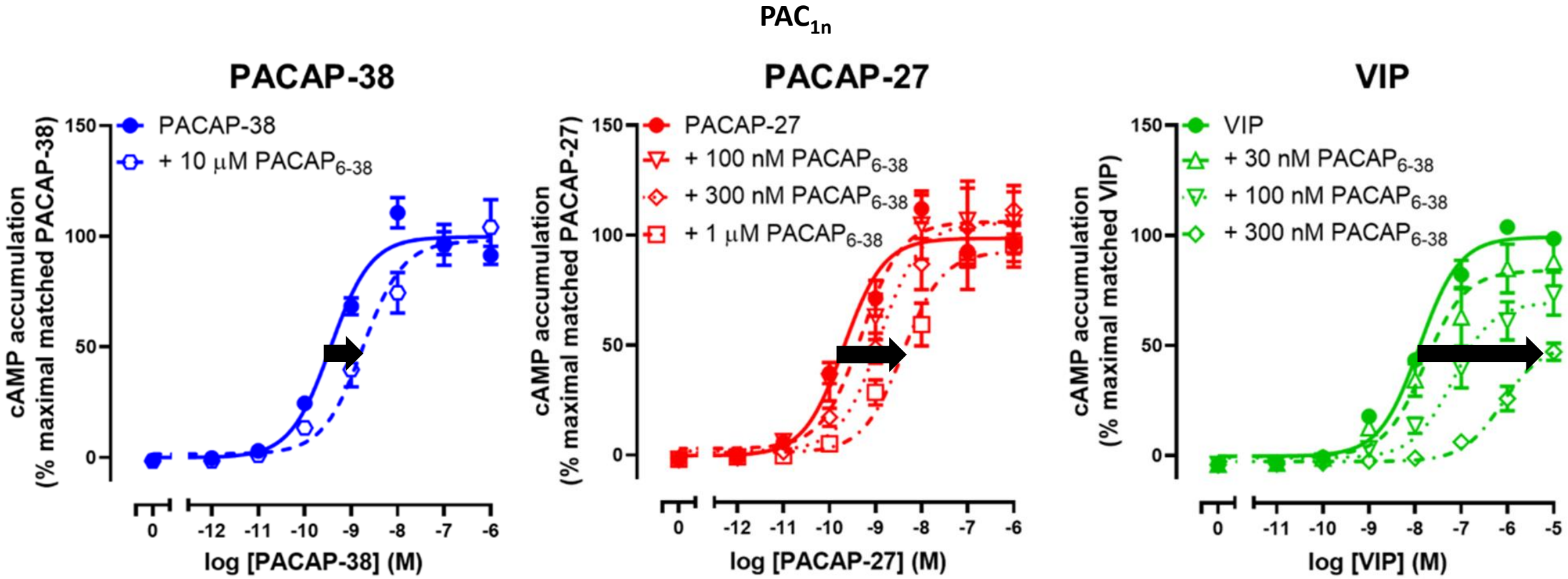








Example Antagonist Data:

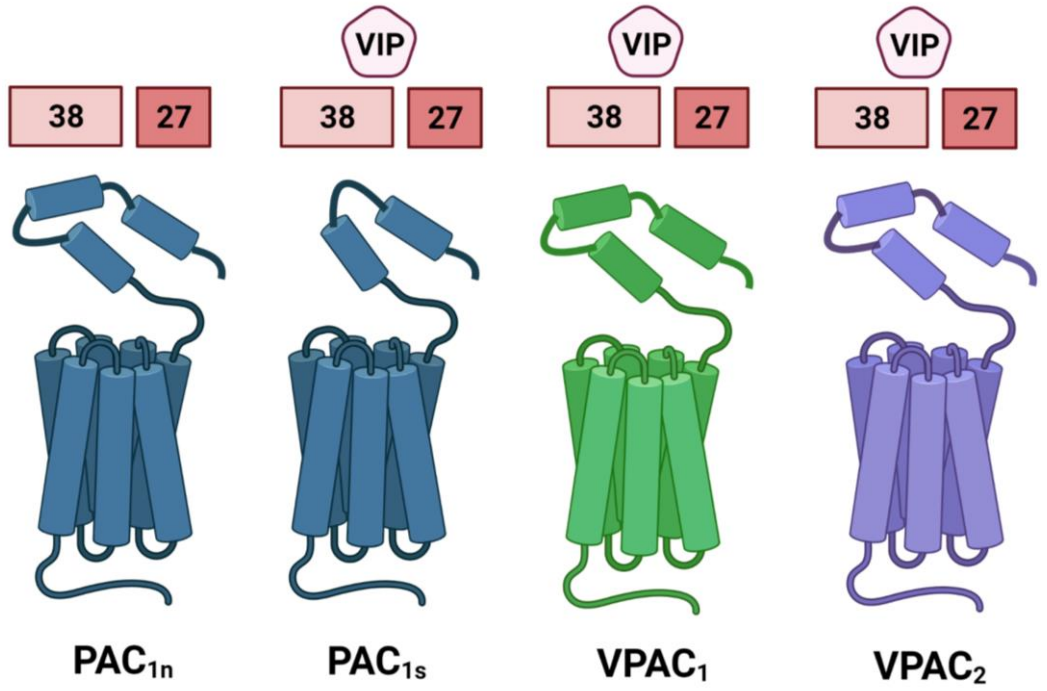


Degree of antagonism depends on the agonist used, with PACAP-38 resistant to antagonism, compared to -27 and VIP

(consistent with prior data in TG neurons Walker et al., British Journal of Pharmacology, 2014.)

Key Conclusions

- Receptors activate multiple signalling pathways
- PACAP is a potent agonist of PAC and VPAC receptors
- VIP <u>can</u> act at PAC_1, not only VPAC receptors
- PAC_{1s} may be a dual receptor for PACAP and VIP
- PACAP-38 is more difficult to antagonize than -27 and VIP



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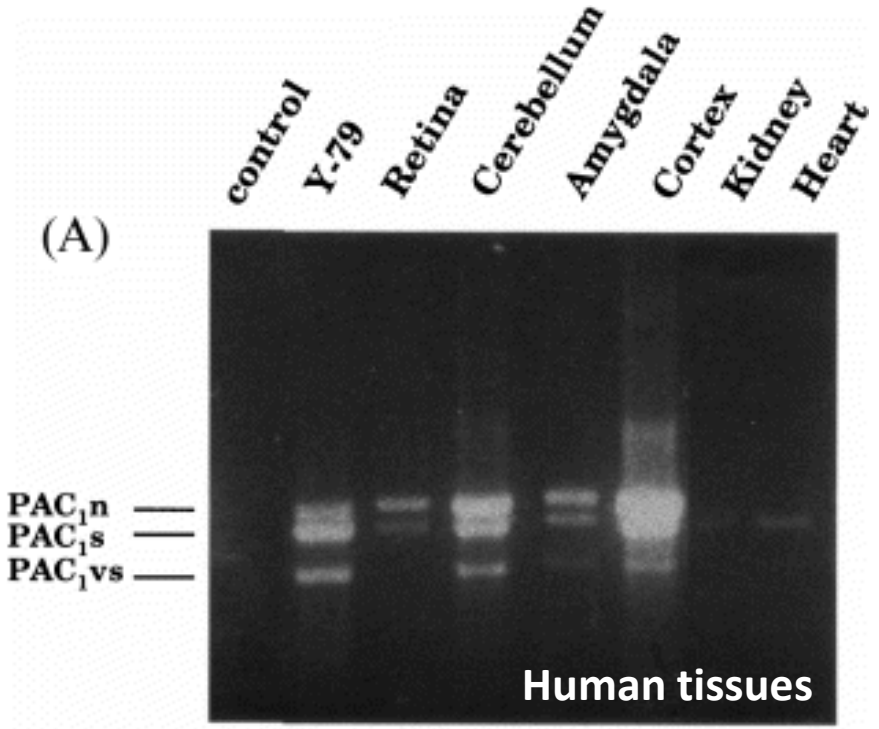
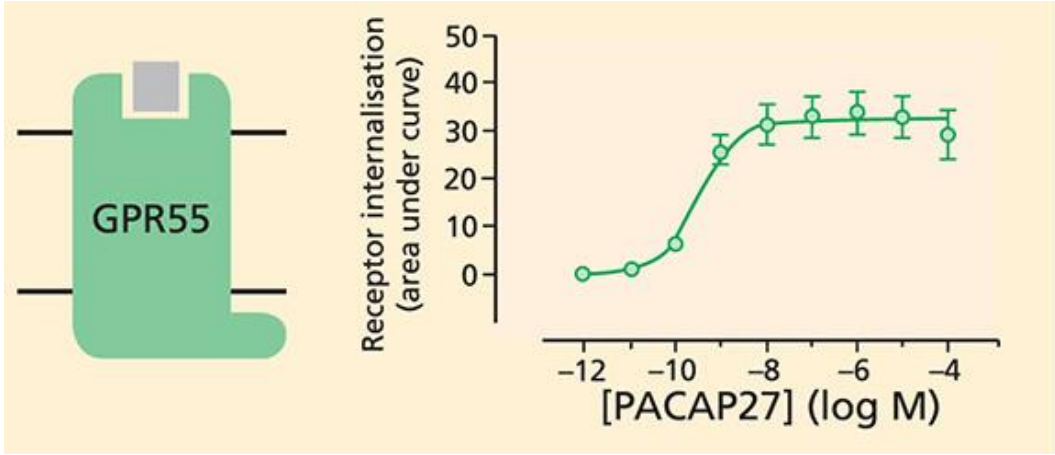
Which Receptor(s)?

- Is PAC_1 ruled out?
- Other splice variants?
- Other receptors?

Development of anti-PACAP/VIP agents for the treatment of migraine needs to consider multiple receptors, multiple ligands & their sites of expression



Dautzenberg *et al.*, *J. Neuroendocrinol*, 1999



Hauser *et al.*, *British Journal of Pharmacology*, 2020
Foster *et al.*, *Cell*, 2019

frontiers in Cellular Neuroscience

PACAP-38 and PACAP(6–38) Degranulate Rat Meningeal Mast Cells *via* the Orphan $MrgB_3$-Receptor

*Sara Hougaard Pedersen[1,2], Sanne Hage la Cour[1,2], Kirstine Calloe[3], Frank Hauser[4], Jes Olesen[1,2], Dan Arne Klaerke[3] and Inger Jansen-Olesen[1,2]**

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