November 2016 AHS Scottsdale

Amylin

(other names – islet amyloid polypeptide [IAPP], diabetes-associated peptide)

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Learning Objectives:

At the end of this presentation you should be able to -

- Define amylin activity
- Define amylin receptors
- Evaluate the importance of amylin receptors in CGRP activity

Presentation outline

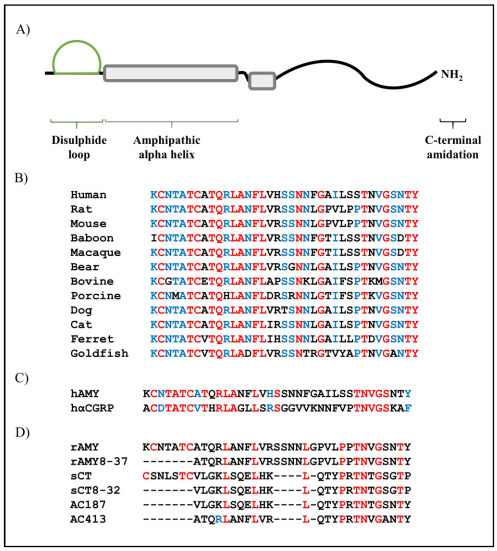
- Amylin: introduction and expression
- Amylin: glucoregulatory and satiety hormone
- Amylin: pain and other actions
- Amylin: receptors and relationship to CGRP receptors
    - Amylin: receptor composition and pharmacology
    - Amylin: receptor binding mechanisms
    - Amylin: receptor expression – is AMY_1 a CGRP or amylin receptor?
- Summary

Amylin: introduction and expression

4

Amylin and CGRP are closely-related peptides

- Both 37 amino acids
- ~40% identical in amino acid sequence
- Share important and highly conserved structural features
  - N-terminal disulfide
  - C-terminal amide
- Some reported activities overlap
- Some receptors overlap



Bower, R.L. & Hay, D.L., 2016 *Brit J Pharmacol*, 173(12):1883-98

5

Amylin expression

- Found in pancreatic islet β cells - co-secreted with insulin
- Also found in stomach, hypothalamus and some neurons

– Note: some "amylin" antibodies can also detect CGRP (see Tingstedt *et al*., 1999, J Histochem Cytochem)

Normal human islets

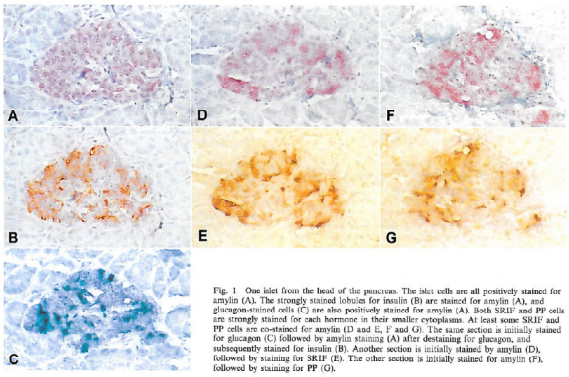
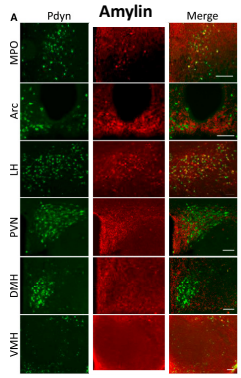


Fig. 1 One islet from the head of the pancreas. The islet cells are all positively stained for amylin (A). The strongly stained lobules for insulin (B) are stained for amylin (A), and glucagon-stained cells (C) are also positively stained for amylin (A). Both SRIF and PP cells are strongly stained for each hormone in their smaller cytoplasms. At least some SRIF and PP cells are co-stained for amylin (D and E, F and G). The same section is initially stained for glucagon (C) followed by amylin staining (A) after destaining for glucagon, and subsequently stained for insulin (B). Another section is initially stained by amylin (D), followed by staining for SRIF (E). The other section is initially stained for amylin (F), followed by staining for PP (G).

Tomita., 2003 *Pathology.* 35:34-36

Rat hypothalamic slices



Li *et al.*, 2015 *Cell Metab. 22*(6):1059-67

6

Amylin expression – trigeminal ganglia (TG) neurons and perivascular fibres

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- Also found in some dorsal root ganglia (DRG) neurons

Cat trigeminal ganglion

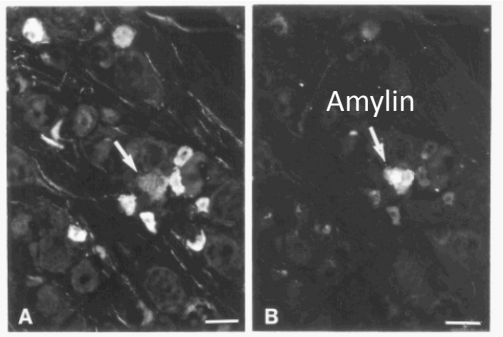

FIGURE 1. Cryostat section through the trigeminal ganglion of the cat showing CGRP-immunoreactivity in scattered nerve cell bodies (A). The same section processed for amylin-immunoreactivity (B) with the double immunostaining method (see Methods). Note identical distribution of cell bodies containing CGRP- and amylin-immunoreactivity (arrows).

Cat pial artery perivascular fibres

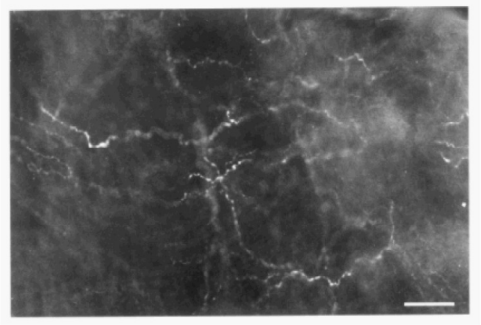

FIGURE 2. Perivascular amylin-immunoreactive nerve fibres in the cat. Whole mount of pial artery (×200).

Edvinsson *et al.*, 2001 *Sci World J. 1*:168-80

7

Amylin expression – brainstem

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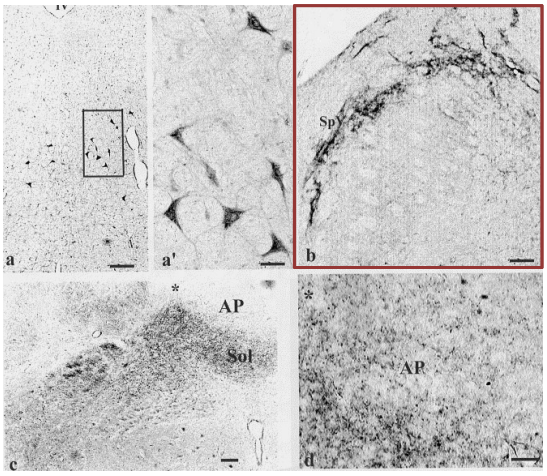
Rat medulla oblongata



Spinal trigeminal tract

D'Este *et al.*, 2000 *Peptides.* 1743-49

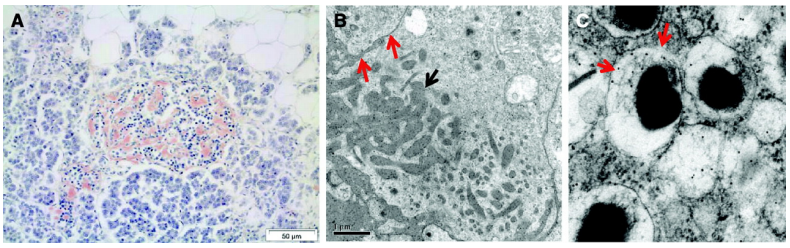
8

Amylin: glucoregulatory and satiety hormone

9

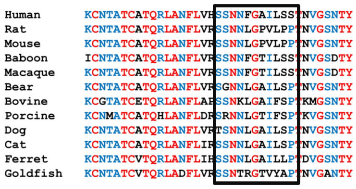
Amylin – physiological role as a glucoregulatory hormone versus pathological role in islet amyloid

- Amylin can form amyloid under some circumstances



Islet amyloid

Westermark *et al.*, 2011 *Physiol. Rev.* 91:795-826



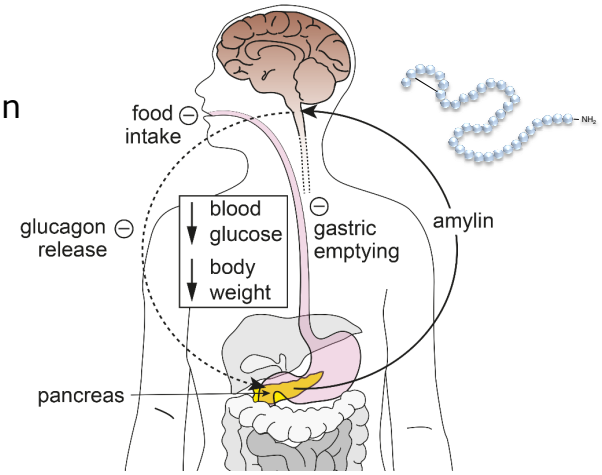
Amyloidogenic region

Bower, R.L. & Hay, D.L., 2016 *Brit J Pharmacol*, 173(12):1883-98

10

Amylin – physiological role

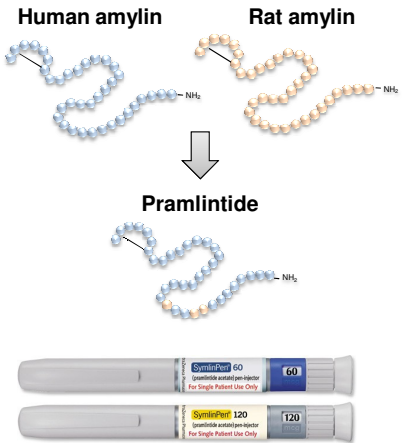
- Co-secreted with insulin from pancreas in response to nutrient intake
- Has a complementary role to insulin in controlling blood glucose
- Deficient in type I and late-stage type II diabetes, where there is β-cell loss



Hay, D. L. *et al.*, 2015 *Pharmacol. Rev.* 67(3):564-600

11

Amylin analogue is an approved therapeutic agent in diabetes

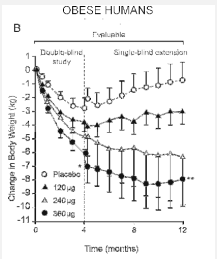


Bower, R.L. & Hay, D.L., 2016 *Brit J Pharmacol*, 173(12):1883-98
Hay, D. L. *et al.*, 2015 *Pharmacol. Rev.* 67(3):564-600

FDA approved — Type I diabetes; insulin adjunct

FDA approved — Insulin-requiring type II diabetes; insulin adjunct

In trials — Obesity



12

Pramlintide reduces food intake and increases "fullness"

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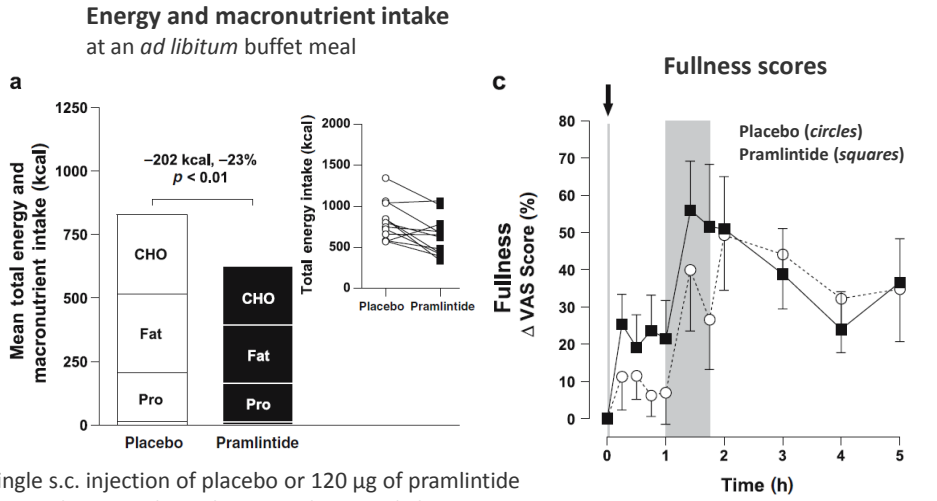

Single s.c. injection of placebo or 120 µg of pramlintide in 11 subjects with insulin-treated type 2 diabetes

Chapman *et al.*, 2005 *Diabetalogia* 48(5):838-48

13

Amylin complements the actions of other metabolic hormones to produce weight loss

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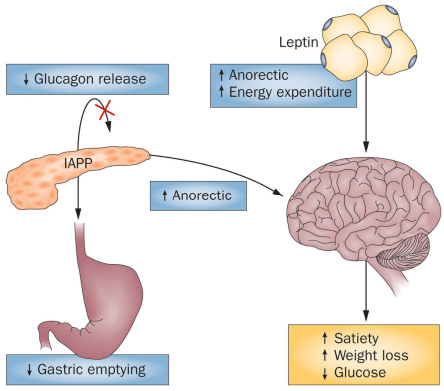
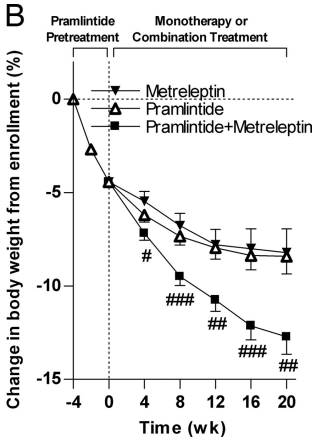
Mechanisms for leptin and amylin co-agonism in the control of body weight and glucose homeostasis



Sadry, S. A. & Drucker, D. J., 2013 *Nat. Rev. Endocrinol.* 9(7):425-33

Human clinical trial of pramlintide in obesity



Roth *et al.*, 2008 *Proc Natl Acad Sci USA* 105:7257-7262

14

Side effects associated with pramlintide use in humans

- Tested in normal human subjects, T1D and T2D, no specific headache cohort
- Severe hypoglycemia

Table 3. Incidence of selected side effects reported during clinical efficacy and safety studies of pramlintide in type 1 diabetes mellitus.

Study	Treatment	n*	Side effects (%)		
			Nausea	Anorexia/reduced appetite	Vomiting
Whitehouse *et al.* 2002 [43]	Placebo	237	21.9	2.1	8.0
52 weeks	30 µg q.i.d.	243	46.5	17.7	11.5
Ratner *et al.* 2004 [71]	Placebo	154	12.0	2.6	6.5
52 weeks	60 µg t.i.d.	164	47.0	18.0	9.8
	60 µg q.i.d.	161	47.0	11.0	11.0
	90 µg t.i.d.	172	59.0	16.0	12.0
Edelman *et al.* 2006 [72]	Placebo	147	36.1	2.0	6.1
29 weeks	30 µg t.i.d. or q.i.d.	41	95.1	14.6	17.1
	60 µg t.i.d. or q.i.d.	101	48.5	6.9	11.9

*Values for n reflect intention-to-treat population.

Younk *et al.*, 2011 *Expert Opin Pharmacother* 12(9):1439-1451

"In a dose-rising tolerability study in non-diabetic volunteers, the highest doses were 10 mg, ~80- to 300-fold higher than anti-diabetic doses. Dose-limiting side effects were nausea and vomiting at 5 mg and 10 mg doses, with no effects reported at doses of 0.3, 1, and 3 mg (Moyses*et al.*, 1993)." From Young, 1995 *Adv. Pharmacol.* 52:289-320

15

Pramlintide and headache?

PubMed search terms: "amylin headache", "amylin migraine", "pramlintide headache", "pramlintide migraine", "symlin headache", "symlin migraine"

- "Treatment with a primed, continuous IV infusion of pramlintide (~16 µg/h) produced consistent mean plasma pramlintide concentrations of 83 ± 3.6, 82 ± 3.7, and 74 ± 3.8 pmol/L"
- 18 "healthy" subjects
- "Back pain, headache, and diarrhea were the 3 most common adverse events accounting for 11% versus 0%, 17% versus 6%, and 11% versus 6% in the pramlintide versus placebo groups, respectively. Those adverse events reported were of mild-to-moderate intensity."

Heise *et al.*, 2004 *Metabolism* 53(9):1227-1232

16

Amylin: pain and other actions

Amylin and pain

- Overall amylin appears to be anti-nociceptive in pharmacology studies (e.g. reduced pain in formalin and acetic acid tests)
- Amylin knockout has anti-nociceptive phenotype
- Calcitonin is anti-nociceptive

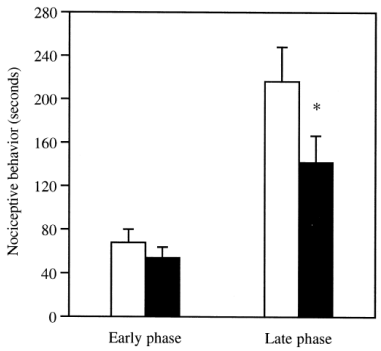


Fig. 2. Nociceptive behavior in the paw formalin test. Nociceptive responses in the early phase (0–5 min) and late phase response (10–30 min) following formalin injection is shown. In the late phase, *IAPP*$^{-/-}$ mice (black bars; $n = 12$) display a shorter duration of nociceptive behavior than wild-type controls (white bars, $n = 10$). Duration of nociceptive behavior in the early phase did not differ between the two groups. Values are means ± S.E.M. Statistical significance was accepted at $P < 0.05$; $* P = 0.027$; Mann–Whitney *U*-test.

Gebre-Medhin *et al., 1998 Brain Res Mol Brain Res 63(1)*:180-3

Other activities of amylin

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TABLE 4
Reported effects of amylin that are not extensively covered in this review and their potential therapeutic area

Potential Therapeutic Area	Key Findings	References
Depression/anxiety	Reduced immobility in forced swim test Increased hippocampal neurogenesis Reduced restraint stress-induced sucrose consumption and hyperthermia Reduced marble burying	Roth et al., 2009; Turek et al., 2010
Memory enhancement	Increased retention under conditions of "weak" conditioning but impaired retention under "strong" conditioning in T-maze Improved learning and memory	Flood and Morley, 1992; Zhu et al., 2015
AD	Decreased brain Aβ levels Improved performance in memory and cognition in preclinical disease models Increased markers of synaptic formation and decreased markers of inflammation and oxidative stress within hippocampus	Adler et al., 2014; Zhu et al., 2015
Antipsychotic/schizophrenia	Intra-accumbens infusion reversed amphetamine-induced prepulse inhibition disruption	Baisley et al., 2014
Pain	Analgesic effects in models of visceral pain when administered peripherally Antinociceptive effects linked to reduced spinal c-Fos expression No effects on tail immersion when given centrally Amylin knockout mice have reduced nociception	Bouali et al., 1995; Gebre-Medhin et al., 1998b; Sibilia et al., 2000; Huang et al., 2010
Osteoporosis	In a streptozotocin (STZ) rat model of diabetic osteopenia, addition of amylin improved bone indices apparently by both inhibiting resorption and stimulating bone formation. Amylin knockout mice have increased bone resorption (decreased bone mass/density, trabecular bone volume) but normal osteoblast and bone formation rates Osteogenic actions depend on diabetic status (effective in low-dose STZ type 2 diabetic but not insulin-resistant preclinical models)	Cornish et al., 1998; Horcajada-Molteni et al., 2000, 2001; Dacquin et al., 2004; Gutierrez-Rojas et al., 2013

From: Hay, D. L. *et al.*, 2015
Pharmacol. Rev. 67(3):564-600

19

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Amylin: receptors and relationship to CGRP receptors

20

Amylin: receptor composition and pharmacology

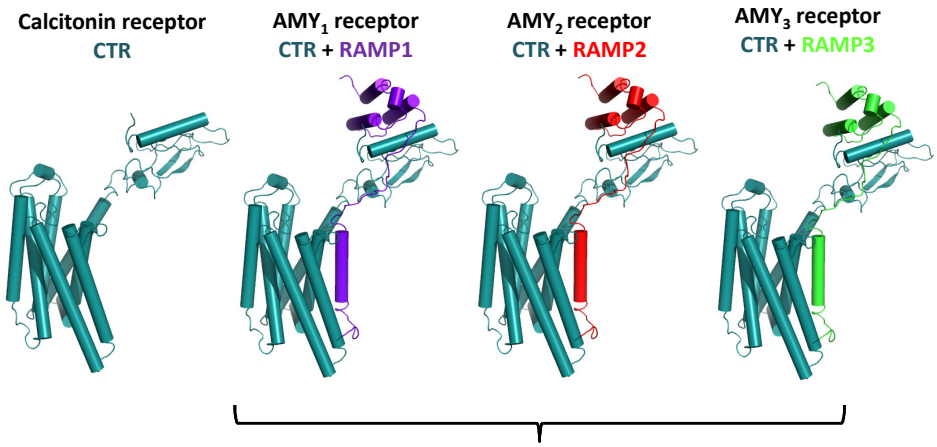
21

Amylin and CGRP receptors

- G protein-coupled receptors (class B)
- For summary see: www.guidetopharmacology.org
- Have different subunits comprising a 7-transmembrane GPCR and a 1-transmembrane accessory protein
  - Calcitonin receptor-like receptor (CLR) - GPCR
  - Calcitonin receptor (CTR) - GPCR
  - Receptor activity-modifying proteins 1, 2 and 3 – accessory protein
  - These combine together to form different subtypes of receptors for the calcitonin peptide family – CGRP, amylin, calcitonin, adrenomedullin, adrenomedullin 2

22

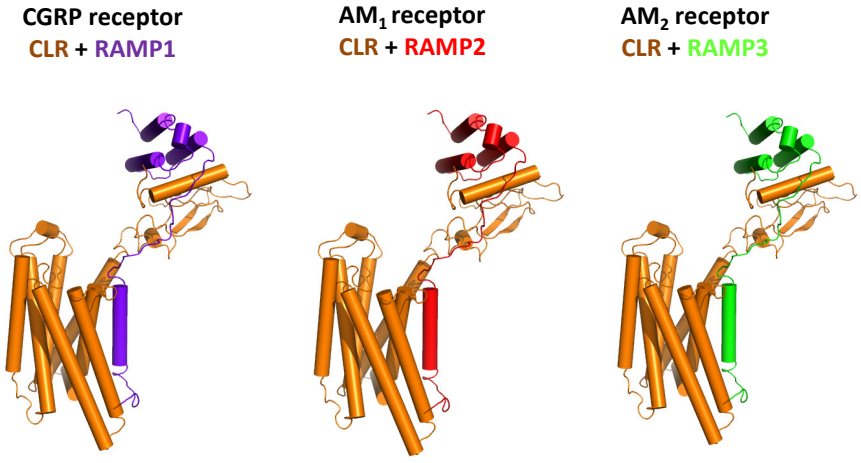
Amylin receptors – composition



23

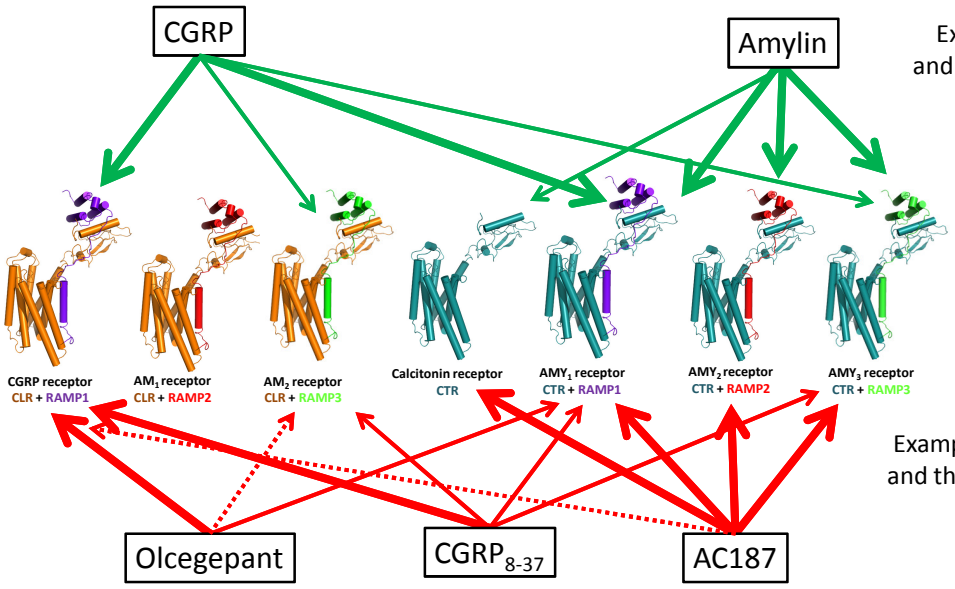
CGRP and adrenomedullin receptors are closely-related to amylin receptors

- Note: the CGRP receptor and AMY_1 receptor share RAMP1



24

There is considerable overlap in pharmacology between receptors

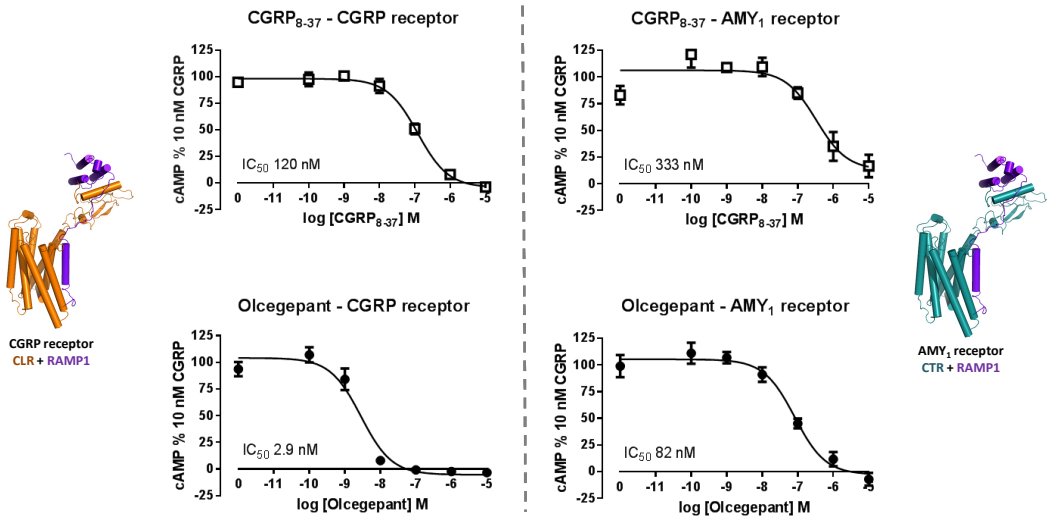


Example agonists and their overlapping activities

Example antagonists and their overlapping activities

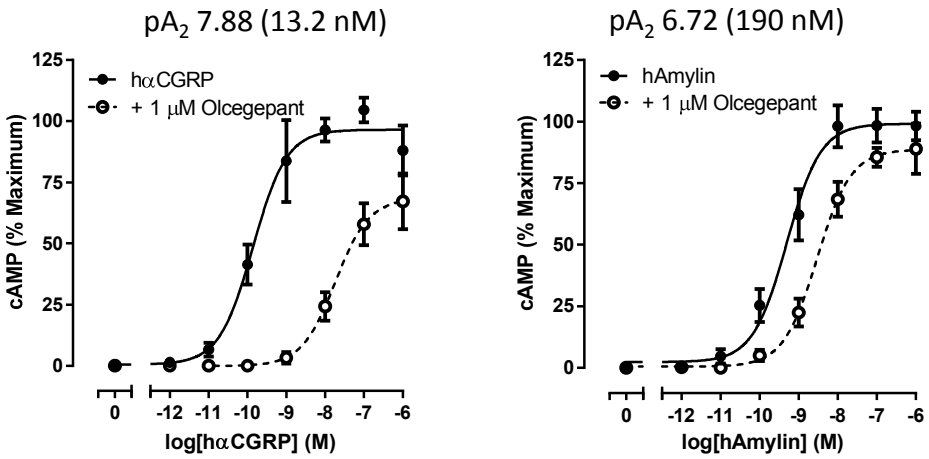
25

CGRP receptor antagonists can fully inhibit CGRP activity at AMY_1 receptors



Hay & Walker, 2016 *Headache*, submitted

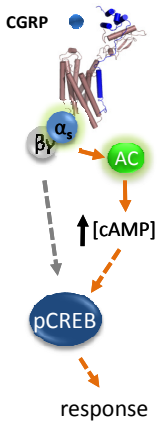
Amylin but not CGRP is resistant to olcegepant (BIBN4096) antagonism at the AMY_1 receptor



Walker, *et al.*, 2016 *Cephalalgia*, in revision

Olcegepant (BIBN4096) antagonism at the AMY_1 receptor depends on the measured activity, further reducing selectivity

	Rat TG neurons	Human CGRP	Human AMY_1	Antagonist selectivity CGRP vs. AMY_1
αCGRP vs. Olcegepant				
cAMP	7.79 ± 0.21 (6) [16.2 nM]	10.00 ± 0.20 (5) [0.1 nM]	7.88 ± 0.12 (5) [13.2 nM]	132
pCREB	9.09 ± 0.04 (4)* [0.8 nM]	10.00 ± 0.24 (6) [0.1 nM]	8.58 ± 0.15 (9)* [2.6 nM]	26
pp38	8.51 ± 0.39 (5) [3.1 nM]	N.D.	N.D.	-
αCGRP vs. Telcagepant				
cAMP	5.72 ± 0.14 (4) [1.9 μM]	8.92 ± 0.05 (5) [1.2 nM]	7.37 ± 0.12 (5) [42.7 nM]	35
pCREB	N.D.	8.71 ± 0.04 (5) [1.9 nM]	7.69 ± 0.32 (5) [20.4 nM]	10

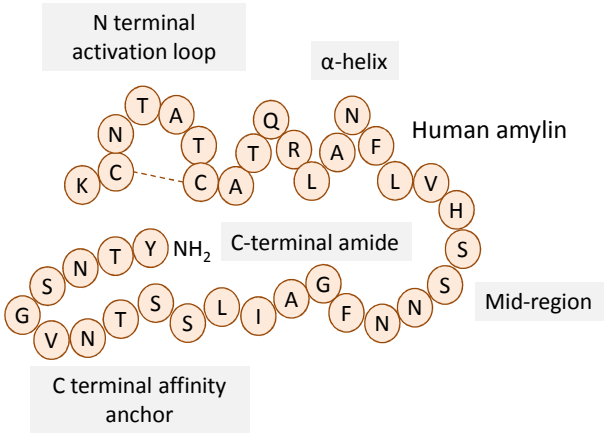


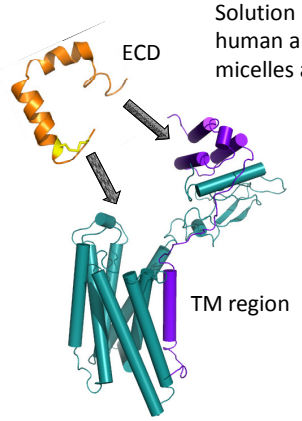
Walker, *et al.*, 2016 *Cephalalgia*, in revision

Amylin: receptor binding mechanisms

29

Amylin – structural motifs and receptor binding mechanism





2L86
Solution NMR structure of human amylin in SDS micelles at pH 7.3

AMY_1 receptor
CTR + RAMP1

30

Nanga, R.B. *et al.*, 2011 *Biochem. Biophys. Acta* 1808:2337-2342

Summary of human amylin alanine scan data

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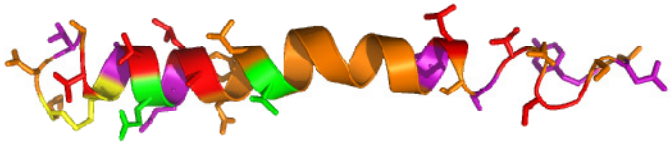
Human	KCNTATCATQRLANFLVHSSNNFGAILSSTNVGSNTY
Rat	KCNTATCATQRLANFLVRSSNNLGPVLPPTNVGSNTY
Mouse	KCNTATCATQRLANFLVRSSNNLGPVLPPTNVGSNTY
Baboon	ICNTATCATQRLANFLVRSSNNFGTILSSTNVGSDTY
Macaque	KCNTATCATQRLANFLVRSSNNFGTILSSTNVGSDTY
Bear	KCNTATCATQRLANFLVRSGNNLGAILSPTNVGSNTY
Bovine	KCGTATCETQRLANFLAPSSNKLGAIFSPTKMGSNTY
Porcine	KCNMATCATQHLANFLDRSRNNLGTIFSPTKVGSNTY
Dog	KCNTATCATQRLANFLVRTSNNLGAILSPTNVGSNTY
Cat	KCNTATCATQRLANFLIRSSNNLGAILSPTNVGSNTY
Ferret	KCNTATCVTQRLANFLIHSSNNLGAILLPTDVGSNTY
Goldfish	KCNTATCVTQRLADFLVRSSNTRGTVYAPTNVGANTY

Bower, R.L. & Hay, D.L., 2016 *Brit J Pharmacol,* 173(12):1883-98



No effect
>10-fold decrease EC_{50}
<10-fold decrease EC_{50}
Increase EC_{50}
Cysteine – not mutated

31

Bower, Yule *et al.*, unpublished

A shared CGRP binding site in the AMY_1 and CGRP receptor extracellular domains?

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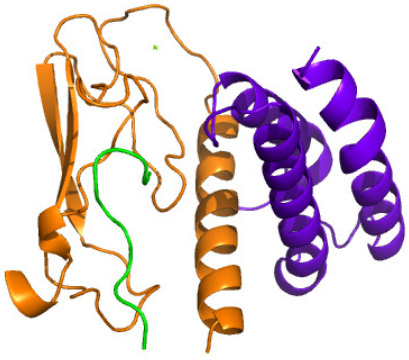
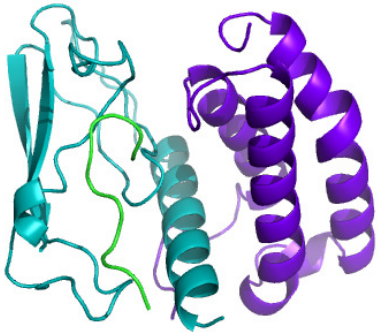
CGRP receptor
CLR + RAMP1, CGRP

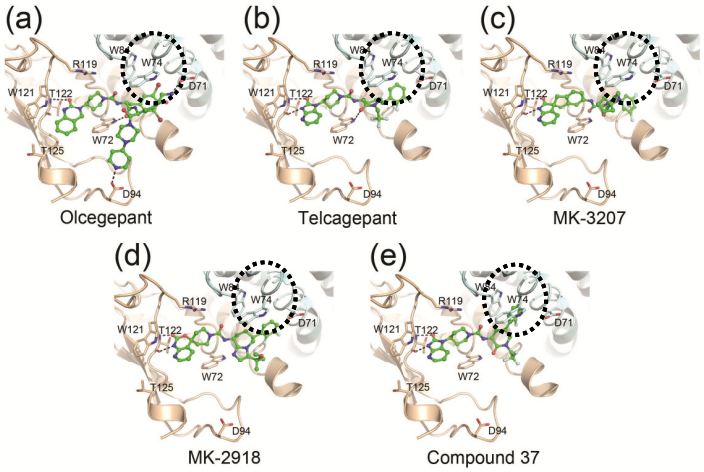


AMY_1 receptor
CTR + RAMP1 , CGRP



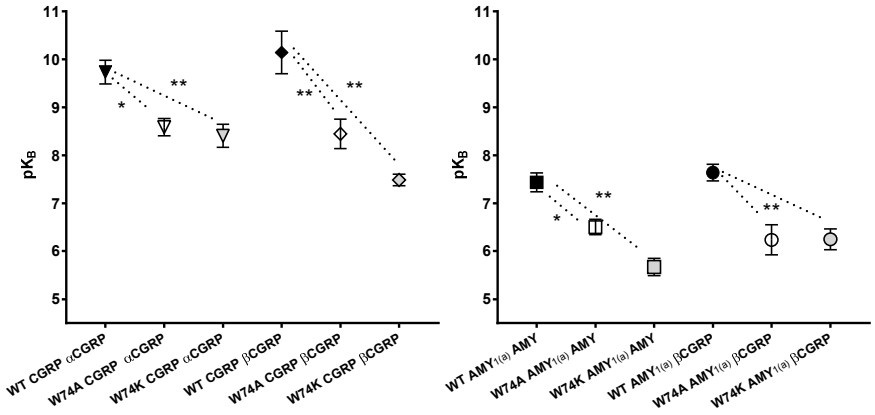
Gingell *et al.*, 2016 *Cell Discovery* 2:16012
Booe *et al.*, 2015 *Mol. Cell.* 58(6):1040-52

Small molecule antagonist binding to the CGRP receptor uses W74 in RAMP1, which is shared with AMY_1



Archbold *et al.*, 2011 *Trends Pharmacol, Sci.* 32(10):591-600

Olcegepant uses the same key RAMP1 residue to bind both AMY_1 and CGRP receptors



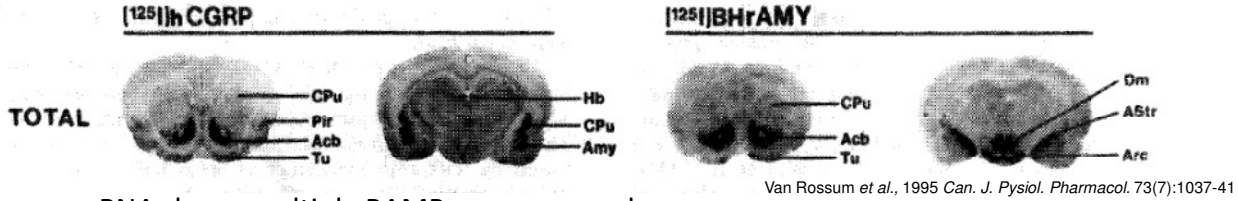
Data replotted from Hay *et al.*, 2006 *Mol Pharmacol*, 70(6), 1984-1991

Amylin: receptor expression – is AMY_1 a CGRP or amylin receptor?

35

Tissue expression of amylin receptors

- Largely unknown
  - Amylin binding shows widespread expression in the brain with some CGRP overlap



Van Rossum *et al.*, 1995 *Can. J. Pysiol. Pharmacol.* 73(7):1037-41

  - mRNA shows multiple RAMPs co-expressed
  - Pharmacological tools cannot distinguish receptors
  - Limited CTR/RAMP1 antibody data; no reliable RAMP2 or RAMP3 antibodies
  - CTR splice variants (also CGRP-responsive e.g. Qi *et al.*, 2013 *Brit. J. Pharmacol*. 168:644-657)
- Not known which receptor(s) mediate amylin action *in vivo* and are the target for pramlintide
- Evidence for AMY_1 as an amylin receptor vs CGRP receptor?

36

Summary of amylin-related transgenic and knockout models

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TABLE 3
Summary of amylin-related transgenic and knockout models

Model	Genetic Background and Method	Reported Effects	Reference
Amylin gene deletion	129Ola/B6 (coding sequence of exon 3)	Lower bone mass/increased osteoclasts	Gebre-Medhin et al., 1998a,b
		Some reduced nociception	
		Increased insulin secretion/more rapid glucose elimination	
		Transient weight gain	
		Reduced sensitivity to anorexigenic effects of CCK	
	Above mice backcrossed onto C57/Bl6	Transient increase in adiposity in females	Turek et al., 2010
		Food intake, body weight unchanged (versus wild type)	
		Reduced sensitivity to endogenous leptin	
		Reduced hypothalamic leptin receptor mRNA	
Amylin overexpression	FVB/n (pronuclear microinjection of construct into fertilized oocytes)	Diabetic	Wong et al., 2008
		Slight decrease in body weight (likely due to glycosuria, consequence of diabetes)	
Calcitonin receptor deletion	Unclear. Deletion of exons 6 and 7 of *calcr*	High bone mass, increased bone formation (normal resorption)	Dacquin et al., 2004
	C57/Bl6. Cre-*Lox*P deletion of exons 13 and 14	Food intake and body weight not well evaluated	Davey et al., 2008
	C57/Bl6. Cre-*Lox*P deletion of exons 6 and 7	Increased bone formation	Keller et al., 2014
RAMP2 and RAMP3 knockout models		RAMP2 deletion is lethal; haploinsufficiency results in defects in bone homeostasis	Dackor et al., 2007; Kadmiel et al., 2011
		RAMP3 knockout models have reduced body weight with normal food intake	
Neuronal RAMP1 overexpression	Multiple lines evaluated	Decreased body weight, adiposity and endogenous leptin	Zhang et al., 2011
		Increased energy expenditure and sympathetic tone	
		Increased BAT, UCP1, and UCP3	
		Enhanced sensitivity to exogenous amylin	

37

Hay, D. L. *et al.*, 2015 *Pharmacol. Rev.* 67(3):564-600

RAMPs affect the activity of other receptors

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Table 1 Summary of RAMP partners and functional consequences

GPCR	GPCR class	Interacting RAMP	Consequence of interaction	Validation in vivo	Supporting reference(s)
GPR30 (an estrogen receptor)	A	RAMP3	Receptor trafficking?	RAMP3-dependent cardioprotection	28
Calcitonin-like receptor (adrenomedullin/ CGRP receptors—CGRP, AM_1, AM_2)	B	RAMP1–3	Receptor trafficking (chaperone, internalization, and recycling), pharmacology	RAMP1, RAMP2, and RAMP3 mouse models linked to adrenomedullin and CGRP biology	4, 34, 41, 101
Calcitonin receptor (AMY_1, AMY_2, AMY_3)	B	RAMP1–3	Pharmacology, modulates signaling	*RAMP1 TG* phenotype associated with amylin function	4, 39
PTH_1	B	RAMP2	Unknown	Unknown	5
PTH_2	B	RAMP3	Unknown	Unknown	5
$VPAC_1$	B	RAMP1–3	Modulates signaling	Unknown	5
$VPAC_2$	B	RAMP1–3	Modulates signaling	Unknown	6, 25
CRF_1	B	RAMP2	Receptor trafficking (chaperone), modulates signaling	Plasma ACTH response	6
Glucagon	B	RAMP2	Unknown	Unknown	5
Secretin	B	RAMP3	Receptor trafficking (rescues mutant receptor)	Unknown	72
Calcium-sensing receptor	C	RAMP1 and -3	Receptor trafficking (chaperone)	Unknown	26, 27

Abbreviations: ACTH, adrenocorticotrophic hormone; AM, adrenomedullin; AMY, amylin; CGRP, calcitonin gene-related peptide; PTH, parathyroid hormone; RAMP, receptor activity-modifying protein; VPAC, vasoactive intestinal peptide/pituitary adenylate cyclase-activating peptide.

38

Hay & Pioszak 2016, *Ann. Rev. Pharmacol. Toxicol.* 56:469-87

CTR expression in the brain

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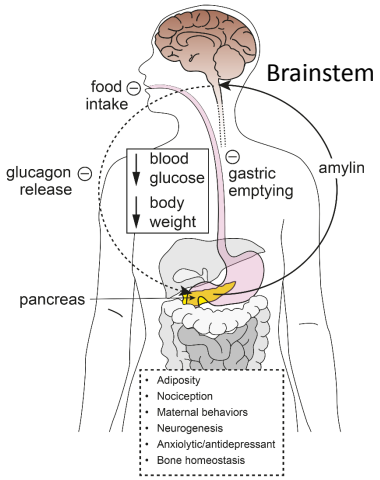
Core subunit:
Calcitonin receptor
CTR

9B4 mAb, HEK293S cells

Walker, C.S. *et al.*, 2015 *Ann. Clin. Transl. Neurol.* 2(6):595-608



Hay, D. L. *et al.*, 2015 *Pharmacol. Rev.* 67(3):564-600

39

CTR in human brainstem

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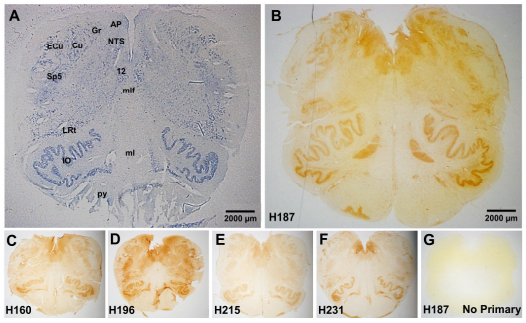
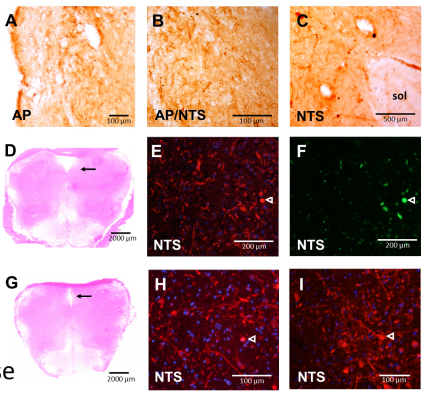
Nissl CTR- 9B4

NTS = nucleus of the solitary tract
AP = area postrema





8 human cases, 65-82 yr, no neurological disease

Walker, C.S. *et al.*, 2015 *Ann. Clin. Transl. Neurol.* 2(6):595-608
Bower, R.L. *et al,*. 2016 *Am. J. Physiol. – Reg. Int. Comp.Physiol.* 310(9):R788-93

40

Functional AMY_1 receptors in TG and brainstem but where else?

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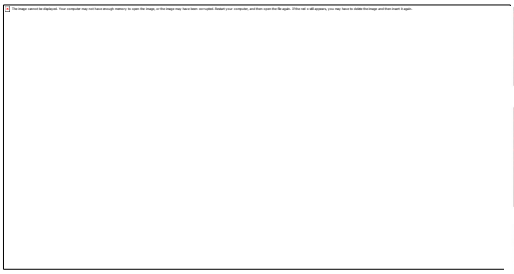
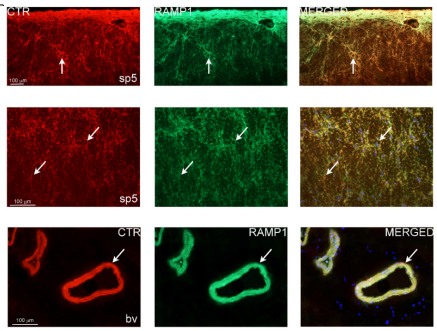
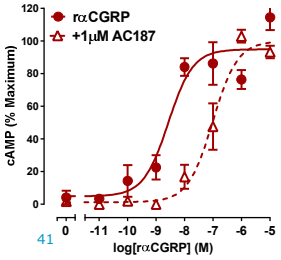
Rat TG





Human brainstem

Rat TG neurons



- CTR + RAMP1 are co-expressed in rat and human TG and human brainstem
- Receptor signals in TG

Walker *et al.*, *Annals of Clinical and Translational Neurology*, 2(6): 595–608, 2015

41

Summary

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- Amylin is natural glucoregulatory hormone that controls satiety
- Amylin and CGRP have overlapping sequences, structures, effects and receptors
- Amylin and CGRP have complex multi-subunit receptors
- The AMY_1 receptor is a CGRP receptor
- CGRP could act through this receptor in migraine
- Many "CGRP receptor" antagonists are also AMY_1 antagonists
- The role of CGRP and AMY_1 receptors in CGRP action needs further defining to inform the safety and efficacy of anti-CGRP classes of drug

42