Calcitonin, amylin, CGRP and adrenomedullin

Overview: Calcitonin (CT), amylin (AMY), calcitonin gene-related peptide (CGRP) and adrenomedullin (AM) receptors (nomenclature as agreed by NC-IUPHAR Subcommittee on CGRP, AM, AMY and CT receptors, see Poyner *et al.*, 2002; Hay *et al.*, 2008) are generated by the genes *CALCR* (which codes for the CT receptor, ENSG00000064989) and *CALCRL* (which codes for the CT receptor-like receptor, CL receptor, previously known as CRLR, ENSG00000004948), whose function and pharmacology are altered in the presence of RAMPs (receptor activity-modifying protein). RAMPs are single TM domain proteins of *ca* 130 amino-acid, identified as a family of three members: RAMP1 (ENSG00000132329), RAMP2 (ENSG00000131477) and RAMP3 (ENSG00000122679). There are splice variants of the CT receptor; these in turn produce variants of the AMY receptor (see Poyner *et al.*, 2002). The endogenous agonists are the peptides CT, αCGRP (also known as CGRP-I), βCGRP (also known as CGRP-II), AMY (also known as islet-amyloid polypeptide, diabetes-associated polypeptide) and AM. There are species differences in peptide sequences, particularly for the CTs. AM2/Intermedin (AM2/IMD) can also activate CGRP, AM_1, AM_2 and AMY_1 receptors, albeit less potently than the cognate agonists (Ogoshi *et al.*, 2003; Roh *et al.*, 2004; Hay *et al.*, 2005). CT receptor-stimulating peptide is another member of the family with selectivity for the CT receptor; it has not been found in humans (Katafuchi *et al.*, 2003). BIBN4096BS is the most selective antagonist available, having a high selectivity for CGRP receptors, with a particular preference for those of primate origin. CGRP-(8-37) acts as an antagonist of CGRP (pK_i 6.5–8.0) and inhibits some AM and AMY responses (7.0). It is inactive at CT receptors. Salmon calcitonin-(8-32) is an antagonist at both AMY and CT receptors. AC187, a salmon CT analogue, is also an antagonist at AMY and CT receptors. Human AM-(22-52) has some selectivity towards AM receptors, but with modest potency, limiting its use.

When co-expressed with RAMP2, the CL receptor produces an AM receptor (AM_1). RAMP3 interacts with the CL receptor to give another receptor that is responsive to AM (AM_2, Fraser *et al.*, 1999). There is some evidence that these AM receptors are pharmacologically distinct (Hay *et al.*, 2003).

Nomenclature	CGRP	AM_1	AM_2
Composition	*CALCRL + RAMP1*	*CALCRL + RAMP2*	*CALCRL + RAMP3*
Principal transduction	G_s/G_q	G_s	G_s
Rank order of potency	CGRP > AM ≥ AM2/IMD > AMY ≥ salmon CT	AM >> CGRP, AM2/IMD > AMY > salmon CT	AM ≥ CGRP, AM2/IMD > AMY > salmon CT
Selective agonists	αCGRP	AM	AM
Selective antagonists	BIBN4096BS (11, Doods *et al.*, 2000; Hay *et al.*, 2003; 2006), MK0974 (8.9, Salvatore *et al.*, 2008)	AM-(22–52) (7, Hay *et al.*, 2003)	–
Probes	[^{125}I]-αCGRP (0.1 nM)	[^{125}I]-AM (rat, 0.1–1.0 nM)	[^{125}I]-AM (rat, 0.1–1.0 nM)

Transfection of $hCT_{(a)}$ with any RAMP can give a receptor with a high affinity for both salmon CT and AMY and varying affinity for different antagonists (Christopoulos *et al.*, 1999; Hay *et al.*, 2005; 2006). $hCT_{(a)}$–RAMP1 [i.e. the $AMY_{1(a)}$ receptor] has a high affinity for CGRP, unlike $hCT_{(a)}$–RAMP3 [i.e. $AMY_{3(a)}$ receptor] (Christopoulos *et al.*, 1999; Hay *et al.*, 2005). However, AMY receptor phenotype is RAMP-type- and cell-line-dependent (Tilakaratne *et al.*, 2000).

Nomenclature	Calcitonin (CT)	AMY_1	AMY_2	AMY_3
Composition	*CALCR*	*CALCR + RAMP1*	*CALCR + RAMP2*	*CALCR + RAMP3*
Principal transduction	G_s/G_q	G_s	G_s	G_s
Rank order of potency	Salmon CT ≥ human CT ≥ AMY, CGRP > AM, AM2/IMD	$AMY_{1(a)}$: Salmon CT ≥ AMY ≥ CGRP > AM2/IMD > human CT > AM	Poorly defined	$AMY_{3(a)}$: Salmon CT ≥ AMY > CGRP > AM2/IMD > human CT > AM
Selective agonists	Human CT	AMY	AMY	AMY
Probes	[^{125}I]-CT (salmon, 0.1 nM), [^{125}I]-CT (human, 0.1–1.0 nM)	[^{125}I]-BH-AMY (rat, 0.1–1.0 nM)	[^{125}I]-BH-AMY (rat, 0.1–1.0 nM)	[^{125}I]-BH-AMY (rat, 0.1–1.0 nM)

The agonists described represent the best available, but their selectivity is limited. AM has appreciable affinity for CGRP receptors and some of its effects can be antagonized by CGRP-(8-37). CGRP can show significant cross-reactivity at AMY receptors and some AM receptors. Responsiveness to human CT can be affected by splice variation (at the rat C1b receptor it is very weak, Houssami *et al.*, 1994). Particularly for AMY receptors, relative potency can vary with the type and level of RAMP present and can be influenced by other factors such as G proteins (Tilakaratne *et al.*, 2000). The major splice variant of the calcitonin receptor, $CT_{(a)}$ has been used for defining the pharmacology of AMY receptors, hence $AMY_{1(a)}$, etc. (see Poyner *et al.*, 2002 for a full description).

G_s is a prominent route for effector coupling but other pathways (e.g. Ca^{2+} and nitric oxide), and G proteins can be activated. The coupling can be affected by splice variants of the CT receptor [e.g. the 490 amino-acid form of the human receptor, $CT_{(b)}$, does not cause an increase in intracellular Ca^{2+} and might have low efficacy in generating cAMP]. There is evidence that CGRP-RCP (a 148 amino-acid hydrophilic protein, ENSG00000126522) is important for the coupling of the CL receptor to adenylyl cyclase (Evans *et al.*, 2000).

[^{125}I]-Salmon calcitonin is the most common radioligand for CT receptors but it has high affinity for AMY receptors and is also poorly reversible. [^{125}I]-Tyr^0-CGRP is widely used as a radioligand for CGRP receptors.

$CGRP_1$ and $CGRP_2$ subtypes have been proposed on the basis of the action of the agonists [Cys(ACM)^{2,7}]CGRP or [Cys(Et)^{2,7}]CGRP (putative $CGRP_2$-selective agents) and antagonist CGRP-(8-37) ($CGRP_1$-selective, pK_i 7.0–8.0, Juaneda *et al.*, 2000). *CALCL/RAMP1* represents the $CGRP_1$ subtype, previously described in native tissues and cell lines (Aiyar *et al.*, 1996; McLatchie *et al.*, 1998), which is now known simply as the CGRP receptor (Hay *et al.*, 2008). The $CGRP_2$ receptor is now considered to have arisen from the actions of CGRP at AM_2 and AMY receptors. It is recommended that this term is no longer used (Hay *et al.*, 2008).

Abbreviations: [Cys(ACM)^{2,7}]CGRP, [acetamidomethyl-Cys^{2,7}]CGRP; **[Cys(Et)^{2,7}]CGRP**, [ethylamide-Cys^{2,7}]CGRP; **AC187**, acetyl-[Asn^{30},Tyr^{32}] salmon CT; **BIBN4096BS**, 1-piperidinecarboxamide, *N*-(2-[{5-amino-1-([4-{4-pyridinyl}-1-piperazinyl]carbonyl)pentyl}amino]-1-[{3,5-dibromo-4-hydroxyphenyl}methyl]-2-oxoethyl)-4-(1,4-dihydro-2-oxo-3[2*H*]-quinazolinyl); **MK0974**, *N*-[(3*R*,6*S*)-6-(2,3-difluorophenyl)-2-oxo-1-(2,2,2-trifluoroethyl)azepan-3-yl]-4-(2-oxo-2,3-dihydro-1*H*-imidazo[4,5-b]pyridin-1-yl)piperidine-1-carboxamide

Further Reading

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