

Orphan Bioactive Peptides

NEWLY IDENTIFIED IN THE REGULATED SECRETORY PATHWAY (RSP) USING PEPTIDOMICS

Snapshot peptidomics of the regulated secretory pathway

Neurons and endocrine cells have the regulated secretory pathway (RSP), in which precursor proteins undergo proteolytic processing by prohormone convertase (PC) 1/3 or 2 to generate bioactive peptides. While motifs for PC-mediated processing have been described (R/K-X_n-R/K, n=0, 2, 4, 6), actual processing sites cannot be predicted from amino acid sequences alone. We hypothesized that discovery of bioactive peptides would be facilitated by experimentally identifying signal sequence cleavage sites and processing sites. However, *in vivo* and *in vitro* peptide degradation, which is widely recognized in peptidomics, often hampers processing site determination. To obtain sequence information about peptides generated in the RSP on a large scale, we applied a brief exocytotic stimulus (2 min) to cultured endocrine cells and analyzed peptides released into supernatant using LC-MS/MS. Of note, 387 of the 400 identified peptides arose from 19 precursor proteins known to be processed in the RSP, including nine peptide hormone and neuropeptide precursors, seven granin-like proteins, and three processing enzymes (PC1/3, PC2, and peptidyl-glycine alpha-amidating monooxygenase). In total, 373 peptides were informative enough to predict processing sites in that they have signal sequence cleavage sites, PC consensus sites or monobasic cleavage sites. Several monobasic cleavage sites identified here were previously proved to be generated by PCs. Thus, our approach helps to predict processing sites of RSP precursor proteins and will expedite the identification of unknown bioactive peptides hidden in precursor sequences.

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Identity of the major peptides released on exocytosis (base peak intensities beyond 2E+06 in the Fig. 2 base peak chromatogram)

RT (min)	m/z (obsd)	z	Mr(Calc)	Mass error (ppm)	Base peak intensity	Score	Expect. value	N-term	Sequence	C-term	Precursor	Validation
8.85	622.7892	2	1243.5615	0.27	4.33E+06	74	3.30E-05	QDEMRLELQR	SANSNPAMAPRE	RKAGCKNFFW	SST	
9.72	504.5915	3	1510.7528	-0.09	4.74E+06	48	0.025	Signal	FPKGGQDKSLHN h Sg III, prepro (20-33)	RELSAERPLN	SgIII	
12.55	613.7739	4	2451.0855	0.40	2.78E+06	59	0.0009	IGVGAPGKKR	DMSSDLERDRPHVSMFQAN h Katalcalcin, Met (O)16	C-term	CT	
13.51	639.0115	3	1914.0112	0.76	1.17E+07	89	0.000033	EVEEKRRKK	NAPPEVPPRAAPAPTHV h VGF, prepro (485-503)	RSPQPPPPAP	VGF	
13.59	609.7757	4	2435.0706	1.26	1.29E+07	65	0.00031	IGVGAPGKKR	DMSSDLERDRPHVSMFQAN h Katalcalcin	C-term	CT	
13.68	751.0048	3	2249.9906	0.87	4.65E+06	45	0.03	IGVGAPGKKR	DMSSDLERDRPHVSMFQAN h Katalcalcin (1-19)	AN	CT	
13.79	659.5054	6	3950.9875	0.31	1.96E+07	119	5.40E-09	Signal	APGRPEAQPPLSSEHKEPVGDAVPGKGSAPVRGA h VGF, Prepro(23-62)	RNSEPQDEGE	VGF	
14.01	774.6837	3	2321.0277	0.67	7.48E+06	65	0.00033	IGVGAPGKKR	DMSSDLERDRPHVSMFQAN h Katalcalcin (1-20)	N	CT	
14.42	734.3739	5	3696.8278	1.45	9.82E+06	72	0.00024	Signal	APGRPEAQPPLSSEHKEPVGDAVPGKGSAPV h VGF, prepro (23-59)	RGARNSEPDQ	VGF	
14.94	827.7590	6	4960.4862	1.23	1.58E+07	100	5.10E-07	M	Ac-SDKPDMAEIKFDKSLKKTETQEKNLPLSKETIEQEKQAGES	RKKRGYRDIN	PC2	TMSB4X
14.94	633.3134	2	1264.6122	0.03	2.66E+06	69	0.00022	HKQQLERDPR	VKMALQOEGFD h PC2, prepro (95-105)	C-term	PC2	
15.46	823.2621	6	4933.5229	1.22	5.12E+06	131	3.80E-10	M	Ac-ADKPDMEIASFDKAKLKTETQEKNLPTKETIEQEKRSEIS	C-term	TMSB10	
15.70	640.3438	2	1278.6721	0.74	7.21E+06	28	0.86	LSRSGGWKN	NFVPTNVGSKAF-NH2 h CGRP I / II (26-37)-NH2	GRRRLDQA	CGRP	manual
16.14	787.4012	5	3931.9663	0.84	2.37E+06	59	0.0055	Signal	FPKGGQDKSLHNRELSAERPLNEQIAEEDI h Sg III, prepro (20-54)	KKTYPPENKP	SgIII	
16.30	682.3453	5	3406.6865	1.06	4.07E+06	118	5.70E-09	RDFSPSSAKR	QOETAAEETETRTHLTRVNLESPPGRVW h VGF, prepro (177-206)	RASWGEFQAR	VGF	
17.37	848.4229	4	3389.6600	0.73	4.51E+06	93	1.70E-06	RDFSPSSAKR	<QOETAAEETETRTHLTRVNLESPPGRVW h VGF, prepro (177-206), pGlu1	RASWGEFQAR	VGF	
17.60	762.0490	3	2283.1230	0.95	2.56E+06	95	8.50E-07	GSQDKSLHNR	ELSAERPLNEQIAEEDI h Sg III, prepro (35-54)	KKTYPPENKP	SgIII	
18.64	800.9424	2	1599.8695	0.46	3.70E+06	50	0.011	Signal	VLPAGGGTVLTKMYP	RGNHWAVGHL	GRP	
19.65	632.5773	4	2642.3381	(-1.20)	2.39E+06	(98)	(2.50E-07)	EGSRIIAQKR	ACDTATCVTHRLAGLLSRSGGWKN h CGRP (1-25)	NFVPTNVGSK	CGRP	
19.79	819.3683	2	1752.7753	(-0.79)	4.71E+06	(70)	(4.90E-05)	NPAMAPRERK	AGCKNFFWKTFTSC	C-term	SST	
19.91	881.7797	3	2642.3187	-0.55	2.47E+06	106	8.60E-08	Signal	APFRSALESSPADPATLSEDEARLL h CT / CGRP I / II, Pro (1-25)	LAALVDQYVQ	CT/CGRP	
20.25	577.2836	3	1844.8873	(-1.10)	3.57E+06	(98)	(1.20E-07)	EGSRIIAQKR	h PC2, prepro (26-75)	RSGGWKNF	CGRP	
20.33	711.9954	8	5697.9079	0.89	2.13E+06	44	0.19	Signal	ERPVTNHFVHKGGEDKARQVAEHGFGVRKLPFAEGLYHFYHGLA	KAKRRRSJHH	PC2	manual
20.37	1089.2665	4	4349.0419	-1.15	3.73E+06	112	1.50E-08	EIINSQVQR	VPQGSSDDLQEEQIEQIAKEHLNQSSQETDKLAPV h SgIII, prepro (527-566)	KRFVGPFPKN	SgIII	
20.60	1144.8639	3	3547.6323	(-1.66)	2.29E+06	(62)	(0.00049)	SLLDSPRSKR	CGNLSTCMGTYTODFNKFTFPOTAIGVGAP-NH2 h Calcitonin	GKKRDMSSDL	CT	
20.84	798.3990	5	3902.9996	(-1.10)	5.05E+07	(117)	(2.80E-09)	EGSRIIAQKR	ACDTATCVTHRLAGLLSRSGGWKNFVPTNVGSKAF-NH2 h CGRP I	GRRRLDQA	CGRP	
21.16	830.0130	5	4145.0262	0.58	2.61E+06	95	1.40E-06	EWLKKHDKK	GNKEDYLSKMIRDFINKQADAYVEKGILDKEAEAI h Sg III, prepro (426-461)	KRIYSSL	SgIII	
21.50	714.3090	12	8559.6167	-0.94	3.09E+07	8	2.2	E+02	Intact ubiquitin	C-term	Ubq	standard
21.52	742.1743	5	3705.8346	0.13	2.51E+07	112	2.40E-08	YPGREAQARR	AOEEAEAEERLQEELENYIEHLLRRP h VGF, prepro (586-615)	C-term	VGF	
21.55	893.4781	3	2677.4147	(-0.82)	4.27E+06	69	0.00031	GVAAPFPKAR	RPESALLGSGEAGERLLQQGLAQVEA-NH2 h VGF, prepro (281-306)	GRRQAEATRQ	VGF	
21.90	1139.5341	3	3531.6374	(-1.57)	2.69E+07	(78)	(1.30E-05)	SLLDSPRSKR	CGNLSTCMGTYTODFNKFTFPOTAIGVGAP-NH2	GKKRDMSSDL	CT	
21.93	1158.8690	3	3589.6429	(-1.68)	9.68E+06	(64)	(0.0003)	SLLDSPRSKR	CGNLSTCMGTYTODFNKFTFPOTAIGVGAPG h Sg III prepro (426-468)	KKRDMSSDL	CT	
22.25	833.0936	6	4992.5178	0.02	7.83E+06	177	1.10E-14	WLLKHDKK	GNKEDYLSKMIRDFINKQADAYVEKGILDKEAEAIKRIYSSL	C-term	SgIII	
23.66	1037.1809	6	6217.0396	0.24	3.95E+07	143	2.50E-11	Signal	APFRSALESSPADPATLSEDEARLLAALVDQYVQMKASELEQEQEREGSSLDSPRS	KRCGNLSTCM	CT	
23.69	10130.3493	6	6056.0436	1.41	3.91E+07	156	1.60E-12	Signal	APFRSALESSPADPATLSEDEARLLAALVDQYVQMKASELEQEQEREGSRIIAQ	KRACDTATCV	CGRP	

Amino acid sequence of human Secretogranin II

MAEAKTHWLG AALSLIPLIF LISGAEAASF QRNQLLQKEP DLRLENVQKF PPEMIRALE
 YIENLRQQAH KEESPDPYNP YQGVSVPLQQ KENGDESHLP ERDSLSEEDW MRIILEALRQ
 AENEPQSAPK ENKPYALNSE KNFPMDMSDD YETQQWPERK LKHMQFPPMY EENSARDNPFK
 RTNEIVEEQY TPQSLATLES VFQELGKLTG PNNQKRERMD EEQKLYTDDE DDIYKANNIA
 YEDVVGGEDW NPVEEKIESQ TQEEVRDSKE NIEKNEQIND EMKRSQGQGI QEEDLRKESK
 DQLSDDVSKV IAYLKRNVNA AGSGRLQNGQ NGERATRLFE KPLDSQSIYQ LIEISRNLQI
 PPEDLIEMLK TGEKPNGSVE PERELDLPVD LDDISEADLD HPDLFQNRML SKSGYPKTPG
 RAGTEALPDG LSVEDILNLL GMESAAANQKT SYFPNPNQEQ KVLPRLPYGA GRSRSNQLPK
 AAWIPHVENR QMAYENLNDK DQELGEYLAR MLVKYPEIIN SNQVKRVPQG GSSEDDLQEE
Human Secretogranin II, Prepro (527-566)
 EQIEQAIKEH LNQGSSQETD K LAPVSKRFP VGPPKND DTP NRQYWDEDLL MKVLEYLNQE
 KAEKGREHIA KRAMENM

April 06, 2009, Phoenix Pharmaceuticals

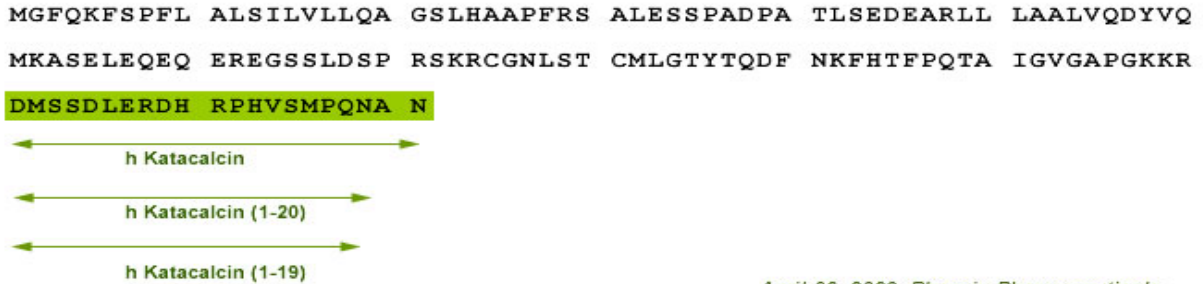
Amino acid sequence of human Secretogranin III

MGFLGTGTWI LVLVLP IQAF PKPGGSQDKS LHNRELSAER PLNEQIAEAE EDKIKKTYPP
 ENKPGQSNYS FVDNLNLLKA ITEKEKIEKE RQSIRSSPLD NKLNVEDVDS TKNRKLIDDY
 DSTKSGLDHK FQDDPDGLHQ LDGTPLTAED IVHKIAARIY EENDRAVFDK IVSKLLNLGL
 ITESQAHTLE DEVAEVLQKL ISKEANNYEE DPNKPTSWTE NQAGKIPEKV TPMAAIQDGL
 AKGENDETVS NTLTLTNGLE RRTKTYSEDN FEELQYFPNF YALLKSIDSE KEAKEKETLI
 TIMKTLIDFV KMMVKYGTIS PEEGVSYLEN LDEMIALQTK NKLEKNATDN ISKLFAPASE
 KSHEETDSTK EEA AKMEKEY GSLKDSTKDD NSNPGGKTDE PKGKTEAYLE AIRKNIEWLK
 KHDKKGNKED YDLSKMRDFI NKQADAYVEK GILDKEEAEA IKRIYSSL

human Secretogranin III, prepro (20-33) human Secretogranin III, prepro (35-54)
 human Secretogranin III, prepro (20-54)
 human Secretogranin III, prepro (426-468)
 human Secretogranin III, prepro (426-451)

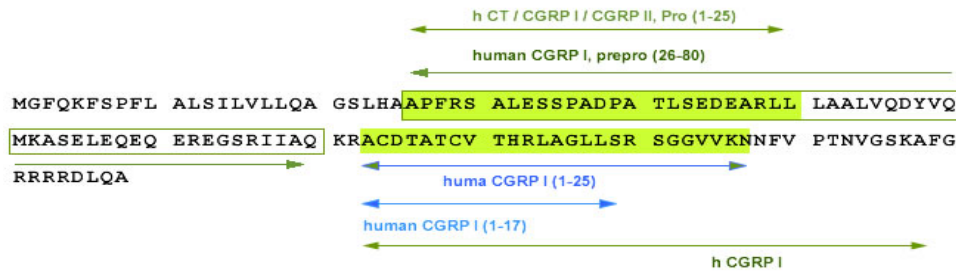
April 06, 2009, Phoenix Pharmaceuticals

Amino acid sequence of human prepro-Calcitonin



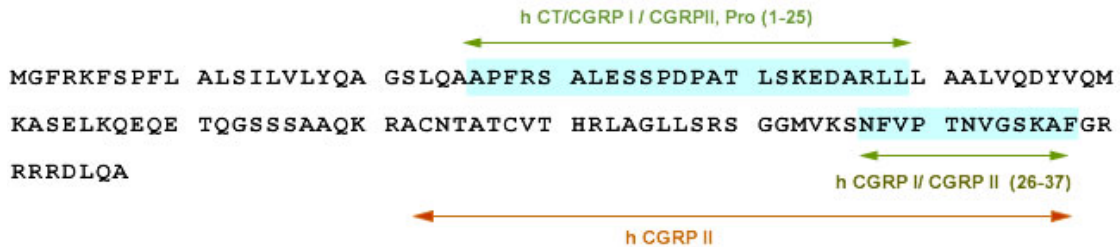
April 06, 2009, Phoenix Pharmaceuticals

Amino acid sequence of human Prepro-Calcitonin-related Peptide I (CGRP I)



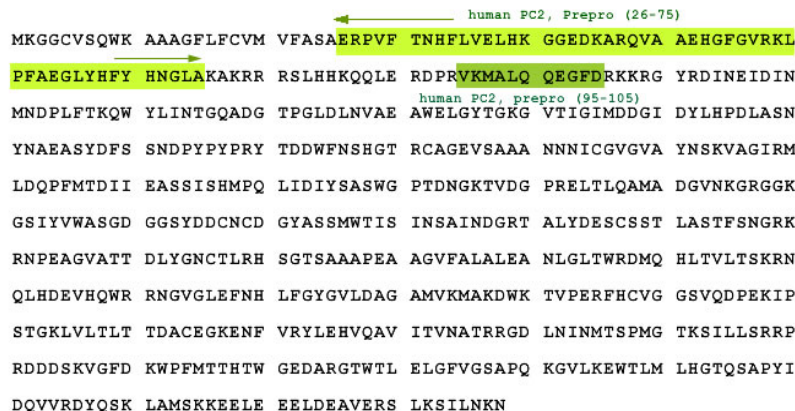
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Amino acid sequence of human Prepro-Calcitonin-related Peptide II (CGRP II)



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Amino acid Sequence of Human PC2



RELATED PRODUCTS

CATALOG NO.	PRODUCT NAME	STANDARD SIZE
007-75	PC2 , prepro (26-75) (Human)	100 ug
014-03	Calcitonin (Human)	500 µg
014-07	Calcitonin, Gly, (Human)	100 ug
014-08	Calcitonin, Met (O)8, (Human)	100 ug
014-41	Calcitonin/CGRP I/ II, Pro (1-25) (Human)	100 ug
015-28	CGRP (1-17) (Human)	200 ug
015-29	CGRP (1-25) (Human)	100 µg
015-02	CGRP (Human)	200 µg
015-30	CGRP I/II (26-37) (Human)	200 ug
015-07	CGRP II (Human)	200 µg
015-31	CGRP, Pro (1-54) (Human)	100 ug
014-32	Katacalcin (1-19) (Human)	200 ug
014-33	Katacalcin (1-20) (Human)	200 ug
014-12	Katacalcin / C-Procalcitonin / PDN-21	500 µg
014-13	Katacalcin, Met (O)16, (Human)	200 ug
007-76	PC2 , prepro (95-105) (Human)	200 ug
004-99	Secretogranin II, prepro (527-566) (Human)	100 ug
004-84	Secretogranin III , prepro (20-33) (Human)	200 ug
004-85	Secretogranin III , Prepro (20-54) (Human)	100 ug
004-86	Secretogranin III , prepro (35-54) (Human)	100 ug
004-98	Secretogranin III , prepro (426-461) (Human)	100 ug
004-87	Secretogranin III , prepro (426-468) (Human)	100 ug
007-67	VEGF, prepro (177-206) (Human, Rat)	100 ug
007-66	VEGF, prepro (23-59) (Human, Rat)	100 ug
007-65	VEGF, prepro (23-62) (Human, Rat)	100 ug
007-69	VEGF, prepro (281-306) (Human, Rat)	100 ug
007-71	VEGF, prepro (485-503) (Human, Rat)	100 ug
007-70	VEGF, prepro (586-615) (Human, Rat)	100 ug

Blast sequences of rat and human VGF

Rat	1	MKTFTLPASV	LFCFLLLIRG	LGAAPPGRSD	30
Human	1	MKALRLSASA	LFC-LLLING	LGAAPPGRPE	29
			h VGF, Prepro (23-62)		
Rat	31	VYPPPLGSEH	NGQVAEDAVS	RPKDDSVPEV	60
Human	30	AQPPPLSSEH	KEPVAGDAVP	GPKDGSAPVE	59
			h VGF, prepro (23-59)		
Rat	61	RAARNSEPDQ	QGELEFQGVDP	RALAAVLLQA	90
Human	60	RGARNSEPDQ	EGELFQGVDP	RALAAVLLQA	89
Rat	91	LDRPASPPAV	PAGSQQGTPE	EAAEALLTES	120
Human	90	LDRPASPPA-	PSGSQQGFEE	EAAEALLTET	118
Rat	121	VRSQTHSLPA	SEIQASAVAP	PRPQTQDNDP	150
Human	119	VRSQTHSLPA	PESPEPA-AP	PRPQTPENGP	147
Rat	151	EADDRSEELE	ALASLLQELR	DFSPSNAKRQ	180
Human	148	EASDPSEELE	ALASLLQELR	DFSPSSAKRQ	177
			h VGF, prepro (177-206)		
Rat	181	QETAAAEETET	RHTLTRVNL	ESPGPERVWR	210
Human	178	QETAAAEETET	RHTLTRVNL	ESPGPERVWR	207
Rat	211	ASWGEFQARV	PERAPLPPSV	PSQFQARMSE	240
Human	208	ASWGEFQARV	PERAPLPPPA	PSQFQARMPD	237
Rat	241	NVPLPETHQF	GEVSSPKTH	LGETLTPLSK	270
Human	238	SGPLPETHKF	GEVSSPKTH	LGEALPLSK	267
			NERP-1		
Rat	271	AYQSLSAPFP	KVRRLGSPFL	GGSEAGERLL	300
Human	268	AYQGVAAFPF	KARPEPESALL	GGSEAGERLL	297
			NERP-2		
Rat	301	QQGLAQVEAG	RRQAEATRQA	AAQEERLADI	330
Human	298	QQGLAQVEAG	RRQAEATRQA	AAQEERLADI	327
Rat	331	ASDLLLQYLL	QAGARQDLG	GRGLQETQQE	360
Human	328	ASDLLLQYLL	QGGARQRLG	GRGLQEAEEE	357
Rat	361	RENEREEEA	QERRGGGEDE	VGEEDDEAAE	390
Human	358	RESAREEEEA	EQERRGGGEER	VGEEDDEAAE	387
Rat	391	AEAEAEAEER	ARQNALLFAE	EEDGEAGAED	420
Human	388	AEAEAEAEER	ARQNALLFAE	EEDGEAGAED	417
Rat	421	KRSQEEAPGH	RRKDAEGTEE	GGEEDDDDEE	450
Human	418	KRSQEEAPGH	RRKEAEGTEE	GGEEDDDDEE	446
Rat	451	MDPQTIDSLI	ELSTKLHLPA	DDVVSIIIEV	480
Human	447	MDPQTIDSLI	ELSTKLHLPA	DDVVSIIIEV	476
			h VGF, prepro (485-503)		
Rat	481	EKRKRKKNNA	PPEPVPPRA	APAPTHVRSP	510
Human	477	EKRKRKKNNA	PPEPVPPRA	APAPTHVRSP	506
Rat	511	QPPP--PAPA	RDELDPWNEV	LPPWDREDE	538
Human	507	QPPPPAPAPA	RDELDPWNEV	LPPWDREDE	536
			TLQP-21		
Rat	539	VFPFGPYHPF	PNYIRPRTLQ	PPASSRRRH	568
Human	537	VYPPGYPYHPF	PNYIRPRTLQ	PPSALRRRH	566
Rat	569	HHALPPARHH	PDLEAQARRA	QEEADAER	598
Human	567	HHALPPSRHY	PGREAQARRA	QEEAEAEER	596
Rat	599	LQEQEELNY	IEHVLLRRP		617
Human	597	LQEQEELNY	IEHVLLRRP		615
			h VGF, prepro (586-615)		

Rat TLQP-21 peptide was identified by Bartolomucci A., et al. PNAS, 2006, 103, 14584-14589
 Nov. 03, 2006, Phoenix Pharmaceuticals, Inc.
 July. 09, 2007, Phoenix Pharmaceuticals, Inc.