Snapshot Peptidomics of the Regulated Secretory Pathway*s

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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. Although motifs for PCmediated processing have been described $((R/K)X_n(R/K))$ where n = 0, 2, 4, or 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 peptide 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-MSMS. 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 α -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. Molecular & Cellular Proteomics 8:1638-1647, 2009.

The generation of peptide hormones or neuropeptides involves the proteolytic processing of precursor proteins by specific proteases. In neurons and endocrine cells, most, if not all, of these bioactive peptides are generated within the RSP¹ in which the processing enzymes PC1/3 or PC2 cleave precursors at basic residues (1, 2). The PC-mediated cleavage most often occurs at consecutive basic residues, but not all basic residues serve as PC recognition sites (2). This is partly because the secondary structure of a precursor also affects the substrate recognition (3). Identification of processing sites is hence a prerequisite for locating unknown peptides hidden in a precursor sequence.

Peptidomics has been advocated to comprehensively study peptides cleaved off from precursor proteins by endogenous proteases (4–6). These naturally occurring peptides are beyond the reach of current proteomics and should be analyzed in their native forms. Unlike proteomics, peptidomics has the potential to uncover processing sites of precursor proteins. Most peptidomics studies, which target tissue peptidomes from brain or endocrine organs (7–11), have provided limited information about secretory peptides that could help to identify processing sites; they are too often blurred by subsequent actions of exopeptidases (cutting off a single amino acid or dipeptide from either end of a peptide).

In MS-based identification of bioactive peptides present in biological samples, their relative low abundance in a total pool of naturally occurring peptides should be considered. Once extracted from cultured cells or tissues, *bona fide* secretory peptides and nonsecretory peptides or peptide fragments caused by degradation of abundant cytosolic proteins cannot be discriminated, and therefore we need to analyze samples rich in secretory peptides to facilitate the identification of bioactive peptides. Several attempts have been made to isolate secretory proteins or peptides, such as subcellular fractionation for harvesting secretory granules (12, 13). With all these efforts, a limited number of secretory peptides still escape analysis.

We took advantage of the fact that peptides processed in the RSP are enriched in secretory granules of neurons and endocrine cells and released on exocytosis. Here we applied a brief exocytotic stimulus (2 min) to cultured human endocrine cells and identified peptides released into supernatant using LC-MSMS on an LTQ-Orbitrap mass spectrometer. Nearly 97% of the identified peptides arose from precursor proteins known to be recruited to the RSP, such as peptide hormone precursors and granin-like secretory proteins. Our

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¹ The abbreviations used are: RSP, regulated secretory pathway; CgA, chromogranin A; CgB, chromogranin B; CT, calcitonin; CGRP, calcitonin gene-related peptide; GRP, gastrin-releasing peptide; PC,

prohormone convertase; SgII, secretogranin II; SgIII, secretogranin III; SST, somatostatin; LTQ, linear trap quadrupole; IPI, International Protein Index.

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approach was validated by the identification of previously known processing sites of peptide hormone precursors. In addition, a majority of the identified peptides retained cleavage sites that agree with consensus cleavage sites for PCs, which are informative enough to deduce the processing sites of RSP proteins. This peptidomics approach will expedite the identification of unknown bioactive peptides.

EXPERIMENTAL PROCEDURES

Peptide Preparation—Monolayer cultures of TT cells (14, 15) were rinsed three times with Hanks' medium (Invitrogen). Culture supernatants of the cells incubated for 2 min before and after stimulation with 10 μM forskolin plus 10 μM carbachol were harvested and rapidly extracted at 4 °C using an RP-1 solid phase extraction cartridge (GL Sciences) without centrifuging the supernatants. Bound substances were eluted in 60% ACN, 0.1% formic acid. After lyophilization of a small aliquot, samples were reconstituted in 10 μl of 2% ACN, 0.1% formic acid. In the one-dimensional analysis, solid phase-extracted analytes were subjected to LC-MSMS without gel filtration using an aliquot equivalent to 5×10^5 cells. Peptide fractions were obtained by HPLC on a gel filtration column equilibrated with 60% ACN, 0.1% TFA at a flow rate of 1.5 ml/min (G2000SWXL, 21.5 × 300 mm, Tosoh Corp.). For gel-filtrated fractions, an aliquot corresponding to 1.5 × 10^6 cells was used for LC-MSMS.

LC-MSMS-Nano-LC-MSMS experiments were performed with a Chorus nanoflow system (CS Analytics) connected to an LTQ-Orbitrap mass spectrometer (ThermoFisher Scientific) equipped with a nanoelectrospray emitter (MonoSpray C_{\rm 18} Nano, 100 $\mu{\rm m}$ \times 50 mm, GL Sciences). Samples were dissolved in solvent A (2% ACN, 0.1% formic acid). The nanoflow system was run at a flow rate of 500 nl/min with a gradient from 5 to 45% solvent B (89% ACN, 0.1% formic acid) in 40 min and then to 95% B in 1 min. A protonated ion of polycyclodimethylsiloxane with m/z 445.120025 was used for internal calibration throughout. The mass spectrometer was operated in a data-dependent mode to automatically switch between MS and MSMS acquisitions. Survey full-scan spectra were acquired in the m/z range 400-1500 with five most intense ions (intensity threshold, 2e+05) sequentially isolated for MSMS in the linear ion trap using collisioninduced dissociation with dynamic exclusion onward throughout the following scans. The resultant product ions were recorded in the Orbitrap.

Data Analysis and Peptide Identification-Peak picking, deisotoping, and deconvolution of MSMS spectra were preformed using Mascot Distiller (version 2.1.1.0) with the default parameters for Orbitrap. Peak lists were searched against IPI human (72,079 entries on July 2, 2008) using Mascot (version 2.2) with no enzyme specification. Pyroglutamination, C-terminal amidation, N-terminal acetylation, and methionine oxidation were simultaneously allowed as variable modifications. Peptide tolerance was set to 2 ppm, and MSMS tolerance was 25 millimass units. The significance threshold was the Mascot default setting of 5%. Each MSMS spectrum was checked manually to confirm or contradict the Mascot assignment. The false discovery rate for the identity threshold was in all cases 0% as estimated by using the Mascot decoy database function. The signals corresponding to intact calcitonin (CT), calcitonin gene-related peptide (CGRP), and somatostatin (SST) (with 1-ppm mass tolerance) underwent a mass shift of 116.01 Da after reductive alkylation with iodoacetamide and were sequenced as such by MSMS in a separate LC-MSMS analysis. Table I lists peptides that were identified with a score above the Mascot homology threshold. In the supplemental table, peptides with a score above the identity threshold (corresponding to an expectation value below 0.05) are listed and were considered identified.



FIG. 1. Gel filtration profiles of culture supernatant extracts from TT cells before (*black* trace) and after stimulation (*gray* trace). *Arrows* indicate molecular mass markers: *A*, 66,500 Da; *B*, 4,271 Da; *C*, 1,673 Da; *D*, 556 Da.

RESULTS

Comprehensive Analysis of Peptides Released on Exocytosis—As a model system, we used the human medullary thyroid carcinoma cell line TT that stores peptide hormones including CT and CGRP in secretory granules (14, 15). A combination of forskolin and carbachol was used to induce exocytosis. Media from cells incubated for 2 min before and after stimulation were separately harvested and solid phaseextracted for peptide analysis. Total peptide amounts were assessed by gel filtration HPLC in which 1000–10,000-Da molecules are eluted in fractions labeled 7–10 (Fig. 1). This exocytotic stimulus elicited a 5.5-fold increase in secreted peptide amounts as assessed by the absorbance at 210 nm.

The solid phase-extracted samples were directly analyzed by LC-MSMS without gel filtration. We first examined a basal level secretion of peptides. In the medium conditioned by TT cells for 2 min, 36 peptides were identified from 13 precursors of which 30 peptides arose from nine secretory proteins including four peptide hormone precursors (CT/CGRP, gastrinreleasing peptide (GRP), and SST), four granin-like proteins (chromogranin A (CgA), chromogranin B (CgB), secretogranin III (SqIII), and VGF), and the processing enzyme PC2 (Fig. 2 and Table I). Because this cell line is known as a hyperproducer of CT and CGRP (14, 16), we tried to locate signals with mass values (within a mass tolerance of 2 ppm from a theoretical value) corresponding to bioactive CT (3415.58 Da) and CGRP (3786.96 Da). Signals from CGRP were observed, but no signals for CT were detected in the base peak chromatogram (Fig. 3).

In contrast, this stimulation facilitated identification of larger numbers of peptides, which resulted in 152 peptides being identified from 18 precursors (Fig. 2 and Table I). The six additional precursors all belonged to secretory proteins known to be processed in the RSP, including three peptide hormone precursors (pituitary adenylate cyclase-activating



Fig. 2. The numbers of peptides identified before and after stimulation sorted by precursor names. CT and CGRP are grouped as they arise from alternatively spliced exons. Peptide sequences are indicated in Table I. *PACAP*, pituitary adenylate cyclase-activating polypeptide; *NPW*, neuropeptide W; *PENK*, proenkephalin A; *KRT*, cytokeratin; *TMSB4X*, thymosin β -4 X-linked; *TMSB10*, thymosin β -10.

polypeptide, neuropeptide W, and proenkephalin A) and three granin-like proteins (7B2, SgII, and pro-SAAS). In total, 146 of 152 peptides arose from the 15 RSP precursors. The remaining six peptides were derived from thymosins and cytokeratin 8. According to the stimulus-induced increase in total peptide amounts released to culture supernatant, more peptides were identified from the former nine precursor proteins (Figs. 1 and 2). Across the CT and CGRP precursor sequences, known major processing products (17) were identified, namely CT N-terminal propeptide (6217.04 Da), bioactive CT (3415.58 Da), katacalcin (2435.07 Da), CGRP N-terminal propeptide (6056.04 Da), and bioactive CGRP (3786.96 Da) (Fig. 3).

Investigation of Cleavage Sites through Identified Peptides-Regarding the processing of peptide hormone precursors, it has long been known that PC1/3 or PC2 cleaves the precursors at sites containing consecutive basic amino acids following N-terminal signal peptide cleavage (1, 2). Cameron et al. (18) recently studied the specificity of PCs and drew a conclusion that the PC-mediated cleavage occurs at sites containing pairs of basic amino acids separated by 0, 2, 4, or 6 residues. However, some peptide hormone precursors are processed at monobasic residues although at much less frequency (2). In any case, the resultant C-terminal basic residues are subsequently removed by carboxypeptidase E. If the exposed C-terminal residue is glycine, peptidyl-glycine α -amidating monooxygenase catalyzes peptide α -amidation, a common post-translational modification often required for a peptide to be fully bioactive (19).

Having confirmed that almost all the sequenced peptides arose from RSP precursors (Table I), we extracted 10 amino

acids N- or C-terminally flanking the sequenced peptides to analyze their cleavage sites. Any glycine immediately followed by a basic residue(s) that creates an amidation site was also counted as a PC consensus site. Monobasic sites were defined as those that do not harbor basic residues except for P1 position and considered potential processing sites as well. PC consensus cleavage sites, signal sequence cleavage sites, and monobasic sites, referred to as informative cleavage sites in the present study, were found in 120, 26, and 29 peptides, respectively (72 peptides were counted twice under this definition). Overall 142 of 152 had such informative cleavage sites at either or both ends.

Identity of Major Peptides in the TT Secretopeptidome-In the LC-MSMS setting used throughout this study, any signal that transcended a given intensity threshold was automatically subjected to MSMS and ignored thereafter if it persisted within a precursor mass tolerance of 5 ppm with the aim of sequencing as many peptides as possible. The signal is not always subjected to MSMS at its maximum intensity in LC-MS profiles, and therefore this setting could return relatively low scores even for abundant peptides, which may not be included in Table I. To identify intense signals in LC-MSMS base peak chromatograms, we examined peptide peak intensities in all MS spectra. Table II provides the list of 35 peptides that were detected at the indicated monoisotopic m/z and charge state with a base peak intensity beyond 2e+06 (see also Fig. 3 and supplemental Fig. 1). Three peptide sequences had expectation values (above 0.05) that did not exceed the Mascot significance threshold. First, the 1278.67-Da peptide was qualified as a CGRP-derived peptide based on the observation that CGRP-derived peptides are most abundantly expressed in the TT secretopeptidome (Fig. 3 and supplemental Fig. 3). Second, the 5687.91-Da peptide also yielded suboptimum MSMS spectra but was identified as a PC2-derived peptide because of matches for eight consecutive b-ions (supplemental Fig. 3). Third, the 8559.61-Da peptide was qualified using MSMS spectral comparison with commercially available human ubiquitin (supplemental Fig. 2).

This list covered all the major processing products of CT and CGRP. Intact CGRP continued to be observed at multiple charged ions (+3 to +6) over a retention time of 10 min in the mass chromatogram, suggesting that it represents one of the most abundant peptides in this secretopeptidome (Fig. 3). The CGRP-derived peptide (ACDTATCVTHRLAGLLSRSGGV-VKN) appeared to be generated from endoproteolytic cleavage of intact CGRP because the C-terminal cleaved half (1278.67 Da) was detected as well (Table II). Except for this peptide, all the N-terminal cleavage sites of CT/CGRP-derived peptides are known as major processing sites (17). Similar findings were obtained with C-terminal cleavage sites except for four non-basic sites. It remains to be clarified whether these non-basic cleavage sites point to the processing that actually occurred in the RSP.

TABLE I

Peptides identified before and after stimulation

Data were obtained without gel filtration chromatography and summarized from three runs using an identical LC-MSMS setting and peptides whose scores (column 7) exceeded homology thresholds (HT, column 8) in at least two runs are listed. For peptides identified in multiple runs, a higher score is listed. Mr(Calc) represents the theoretical monoisotopic molecular mass (Da) based on the peptide sequence. The score value beyond an identity threshold (IT, column 9) is indicated in bold. In column 1, "CT/CGRP" indicates that the peptides are shared by CT and CGRP precursors. If the peptide was identified at different charges, the charge states are also shown in column 4. Expectation values are indicated in column 10. The N- (N-term) and C-terminal (C-term) flanking 10 amino acids (columns 11 and 13) are shown and marked as follows: closed boxes with white letters, typical PC cleavage sites containing consecutive basic residues and sites having C-terminal amidation motifs; dark gray boxes, cleavage sites containing basic residues at P4, P6 or P8 position; pale gray boxes, sites having basic residues at P1 but not at P2, P4, P6 or P8 position. "Signal" indicates that the peptide flanks its signal sequence. "C-term" indicates that the peptide C-terminus is the end of the precursor protein. Ac-, N-terminal acetylation; -NH2, C-terminal amidation; <Q, pyroglutamic acid. Oxidized methionine residues are underlined in column 12. Sequences are based on the following IPI accession numbers: CgA, 00746813; CgB, 00006601, CGRP, 00027855; CT, 00000914; GRP, 00011722; KRT18, 00554788; KRT8, 00554648; PC2, 00029131; SgIII, 00292071; SST, 00000130; TMSB10, 00220827; TMSB4X, 00220828; VGF, 00069058; 7B2, 00008944; NPW, 00853190; PACAP, 00000027; PENK, 00000828; SgII, 00009362. PACAP, pituitary adenylate cyclase-activating polypeptide; NPW, neuropeptide W; PENK, proenkephalin A; KRT, cytokeratin; TMSB4X, thymosin β -4 X-linked; TMSB10, thymosin β -10.

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Sylit 86,9151 2 1731,813 -0,000 F6 2 4 0.000 DEFENDENCE SSPLDNELWOST KMRK TMSER10 22,2413 6 4,5,7 433,852 0.001 66 2 M A-ADRFOMGEAPTOKALKKTET DEINT FREAD C-Amm TMSER10 22,2413 6 4,5,7 433,852 0.001 5 2 M A-ADRFOMGEAPTOKALKKTET DEINT FREAD C-Amm VGF 917,743 4 5 369,9757 -0.000 52 8 3,326.65 Sympl APFORPEAOPPH, SEHKET/VAGDWAPCHOGSAPEV RESENDERS VGF 391,741 4 5 369,9757 -0.000 12 2 4 3,026.65 Sympl APFORPEAOPHYS, SEHKET/VAGDWAPCHOGSAPEV RESENDERS C-Amm VGF 391,471 4 36,46 0.001 12 2 4 3,026.65 Symple APFORPEAOPHYS, SEHKET/VAGDWAPCHOGSAPEV RESENDERS C-Amm VGF 843,4221 4 380,86.680	SgIII	787.4010	5		3931.9663	0.0021	29	16	49	5.4	Signal	FPKPGGSQDKSLHNRELSAERPLNEQIAEAEEDKI	KKTYPPENKP
SST 62,7,780 2 1243,661 0,000 66 2 4 0.0002 DDEMNELOR SANSHYAMAPRE RKACCONFPU RKACCONFPU TMSB1A 427,7746 6 4,5,7 463,522 0,000 78 6 5 4,5,7 460,482 -0,002 75 6 1,462,45 M A-SDRFDMAEREPDRSKLKKTETC GENNPLPKETEGEKAGES C-lem VGF 917,7143 4 366,023 0,000 62 4 0,0007 Synah A-SDRFDMAEREPDRSKLKTETCGENNPLPKETEGEKAGES C-lem VGF 917,7143 4 366,023 0,000 52 0,48 0,00007 Synah A-PPCRPEADPRSKLKTETCENNPLPKETEGEKAGES C-lem VGF 913,011 5 360,000 102 48 0,00007 Synah A-PPCRPEADPRACERTICAGENAR NAPPEVPERAD RSAGEORA RSAGEORA <t< td=""><td>SgIII</td><td>866.9151</td><td>2</td><td></td><td>1731.8163</td><td>-0.0007</td><td>45</td><td>22</td><td>45</td><td>0.06</td><td>KIEKERQSIR</td><td>SSPLDNKLNVEDVDST</td><td>KNRK</td></t<>	SgIII	866.9151	2		1731.8163	-0.0007	45	22	45	0.06	KIEKERQSIR	SSPLDNKLNVEDVDST	KNRK
TASE 10 823,2613 6 5, 7 493,252 0,0012 146 49 8,05-12 M Ac-ADR/PMORES/EDEX/STUPEX/ETTECEXATLPTIKETTECEXATLSES C-lerm TABBA K 27,374 6 4,5,7 490,462 -0.0027 Signal APCARPTIALER/FDEXKIKET CERAPLPSKETTECEXACES C-lerm TABBA K 27,7143 4 5,6 390,487,44 4 5,0 390,476 4 0.00027 Signal APCARPTADERFEXTOR C-lerm VCF 917,714 4 5,6 390,487,47 4 0.00027 Signal APCARPEADPRISSENKEPVA0DAVPGROSSAPEV C-lerm C-lerm VCF 933,740 4 5,6 390,487 4.0006 4 2.001704 1.0 DECEXARVA APPCARPEADPRISSENKEPVA0DAVPGROSSAPEV C-lerm C-lerm VCF 933,714 5 191,6112 0.001 1.0 2 2.001704 1.001795353AR AUCETARARVA APPCARPEADAPTINV MACETARRVA ARSV PHPPADA VCF 852,8792 4 300,658 0.0017 1.00275353AR 2.00171A2ETERHLICEDELWYETHVLERP C-lerm T12 751,82 750,899 0.0015	SST	622.7880	2		1243.5615	0.0000	66	22	41	0.0002	QDEMRLELQR	SANSNPAMAPRE	RKAGCKNFFW
TM8B4X 827.7546 6 4.5.7 490.4862 -0.002 75 50 1.0E-06 M Ac-SDKPMALBEKPDSKLIKKTET GEKNPLPSKETTEQEKAGESS C-kerm VGF 917.7143 4 3669.277 0.0004 50 3.00-05 S Bynl APPCRPEADPH.SSEHKEPVADAVPERKTEQEKAGESS C-kerm VGF 917.7143 4 3669.277 0.0004 90 2.0 4 0.0005 7 Signal APPCRPEADPH.SSEHKEPVADAVPERKGESAPEVKG RRAREPDEVKGESAPEKKGESAPEKKGE	TMSB10	823.2613	6	4, 5, 7	4933.5229	0.0012	148		49	8.50E-12	M	Ac-ADKPDMGEIASFDKAKLKKTET QEKNT LPTKETIEQEKRSEIS	C-term
TNSB4X 996-303 5 997-8411 -0.0002 77 49 9.90E-05 Model State Common State <th< td=""><td>TMSB4X</td><td>827.7546</td><td>6</td><td>4, 5, 7</td><td>4960.4862</td><td>-0.0020</td><td>95</td><td></td><td>50</td><td>1.40E-06</td><td>M</td><td>Ac-SDKPDMAEIEKFDKSKLKKTET QEKNPLPSKETIEQEKQAGES</td><td>C-term</td></th<>	TMSB4X	827.7546	6	4, 5, 7	4960.4862	-0.0020	95		50	1.40E-06	M	Ac-SDKPDMAEIEKFDKSKLKKTET QEKNPLPSKETIEQEKQAGES	C-term
VGF 917.7143 4 3866.2278 0.0006 50 24 40.0005 Signal APPCRPEAOPPR.SEHKEPVAGDAVPCPK0GSAPEV RGARNSEPCD VGF 932.714 4 5 3706.344 0.0006 32 18 48 2.0 VGPREAOPPR.SEHKEPVAGDAVPCPK0GSAPEVKGA FRSRAPPPAP VGF 639.010 3 194.0112 0.0006 2.2 48 4.0000 1.0 2.0 VGPREAOPPR.SEHKEPVAGDAVPCPK0GSAPEVKGA C-4mm VGF 848.4221 4 3306.6860 0.0007 19 2.0 48 4.00004 C-4mm VGPREAOPPR.SENKEPVAGDAVPCPK0GSAPEVW RASVCPEPAR VGF 843.421 4 3306.6860 0.0007 109 2.0 48 4.00002 VGPREAOPPR.SENKEPVAGDAVPCPK0GSAPEVW RASVCPEPAR 712 861.4669 2 1771.9271 0.0000 48 40.0002 VGPREAOPPR.SENKEP VGPREAOPPR.SENKEPVAGDAVPCWORAAPENV AVGKSVPHF3DE 722 861.4669 2 1771.9271 0.0007 40 0.0002 VGGRERRKR	TMSB4X	996.3035	5		4976.4811	-0.0002	77		49	9.90E-05	M	Ac-SDKPDMAEIEKFDKSKLKKTET QEKNPLPSKETIEQEKQAGES	C-term
VGF 988,750 4 5.6 3980,8975 -0,000 90 23 48 2,500 APPCRACHERSARY CLOUD CLOUD <thc< td=""><td>VGF</td><td>917.7143</td><td>4</td><td></td><td>3666.8278</td><td>0.0004</td><td>68</td><td>23</td><td>48</td><td>0.00067</td><td>Signal</td><td>APPGRPEAQPPPLSSEHKEPVAGDAVPGPKDGSAPEV</td><td>RGARNSEPQD</td></thc<>	VGF	917.7143	4		3666.8278	0.0004	68	23	48	0.00067	Signal	APPGRPEAQPPPLSSEHKEPVAGDAVPGPKDGSAPEV	RGARNSEPQD
YGF 742,1746 5 3705,8346 0.0020 32 18 48 2.5 YGF READARR ADAEAEERRI.GEQELLENYIEHVULLRP C.horm RSPOPPPAP YGF 684,4221 4 3386,8600 -0.007 199 20 48 4.00E-08 RSPSPSSAKR ADAEAETERTHTITKVULESPGPERVW RASW GEPOAR After stimulation	VGF	988.7540	4	5,6	3950.9875	-0.0006	90	23	49	3.90E-06	Signal	APPGRPEAQPPPLSSEHKEPVAGDAVPGPKDGSAPEVRGA	RNSEPQDEGE
VGF 6439.110 3 1914.0112 0.0001 32 20 4 1.3 EVEKTRKIKK NAPPEVPEPRAAPARTHV RRSPORPPAPA VGF 648.421 4 3389.660 0.0010 102 24 2.0024 RDFSPSSXKR VOETAAAETETRTHTLTRVNLESPGPERVW RASW GEFOAR After stimulation 782 86.6865 2 1770.921 44 2.5 45 0.002 2MKGSGERRKRR SVNPYLOGORLDNVV AKKSVPHFSDE 782 86.6865 2 1770.921 0.000 64 2.4 0.0022 2MKGGERRKR SVNPYLOGORLDNVV AKKSVPHFSDE 782 751.3260 2 1500.8389 0.0006 64 2.4 0.0022 SMKISFRAR XYGFROPCPQ C-tom C-tom CgA 646.7531 2 196.9996 3 0.0001 65 24 5 0.002 SSMKISFRAR YGFROPCPQL RRGWRPSSRE CgA 666.731 3 199.4996 0.0004 54 60.012 FRGPCPQLR WRPSREDSLEAGLPLQ VROYFEKKE RGROPCPQL RGROPCPQL RGROPCPQL RGROPCPQL </td <td>VGF</td> <td>742.1746</td> <td>5</td> <td></td> <td>3705.8346</td> <td>0.0020</td> <td>32</td> <td>18</td> <td>48</td> <td>2.5</td> <td>YPGREAQARR</td> <td>AQEEAEAEERRLQEQEELENYIEHVLLRRP</td> <td>C-term</td>	VGF	742.1746	5		3705.8346	0.0020	32	18	48	2.5	YPGREAQARR	AQEEAEAEERRLQEQEELENYIEHVLLRRP	C-term
VGF 684.221 4 338.8600 -0.0007 102 20 48 4.006.06 RDSPSPSAKR COCETAAAETETRTHTLTRVNLESPGPERVW RASW GEFOAR After stimulation 782 851.4489 2 1700.8846 -0.0012 44 2.506.47 ROPSPSAKR SVNPYLGGGRLDNVV AAAETETRTHTLTRVNLESPGPERVW AKKS VPH/FSD 782 851.4489 2 1700.8846 -0.0012 44 2.506.47 0.022 AKGSVPH/FSD CALM KKSVPH/FSD 782 751.266 2 1700.8846 -0.0017 68 2.46 0.0022 GRLDNVKAK SVNPYLGGGRLDNVV AKSVPH/FSDE CALM CqA 161.430 0.0016 64 2.46 0.0022 SMIKLSFRAR AYGR PCPCQ CALM CALM RGWPRPSSRE CALM RGWPRPSSRE CALM RGWPRPSSRE CALM RGWPRPSSRE CALM RGWPRPSSRE CALM RGWPRPSSRE CALM CALM RGWPRPSSRE CALM CALM RGWPRPSSRE CALM CALM CALM CALM <td< td=""><td>VGF</td><td>639.0110</td><td>3</td><td></td><td>1914.0112</td><td>0.0001</td><td>32</td><td>20</td><td>46</td><td>1.3</td><td>EVEEKRKRKK</td><td>NAPPEPVPPRAAPAPTHV</td><td>RSPQPPPPAP</td></td<>	VGF	639.0110	3		1914.0112	0.0001	32	20	46	1.3	EVEEKRKRKK	NAPPEPVPPRAAPAPTHV	RSPQPPPPAP
VGF B52.6792 4 9406.6865 0.0010 102 48 2.50E-07 RDFSPSSAKR QQETAAAETETRTHTLTRVNLESPOPERVW RASW GEFOAR After stimulation -	VGF	848.4221	4		3389.6600	-0.0007	109	20	48	4.00E-08	RDFSPSSAKR	<qqetaaaetetrthtltrvnlespgpervw< td=""><td>RASW GEFQAR</td></qqetaaaetetrthtltrvnlespgpervw<>	RASW GEFQAR
After stimulation 782 851,489 2 1700,8846 -0.012 44 25 46 0.083 MKGCERRKRR SVNPYLQGORLDNVA KKSVPHFSDE 782 751,3260 2 1700,8846 -0.0012 44 25 47 0.022 KMGGERRKRR SVNPYLQGORLDNVA KKSVPHFSDE Calem CpA 551,3260 2 1006,550 -0.0007 69 32 45 0.0002 SSMKISFRAR AVGR GPGPQ LRRGWRPSSRE CpA 551,3303 2 1045,5593 0.0001 53 45 0.0002 SSMKISFRAR AVGR GPGPQ LRRGWRPSSRE CpA 666,6731 3 299,6950 0.0001 43 25 47 0.012 FRGFGPQR GRWPSREDSLEAQLPLQ VROWRPSSRE KROWRPSSRE	VGF	852.6792	4		3406.6865	0.0010	102		48	2.50E-07	RDFSPSSAKR	QQETAAAETETRTHTLTRVNLESPGPERVW	RASW GEFQAR
After stimulation XNPYLOGORLDNVV ANKSVPHFSD ANKSVPHFSD 782 886,988 2 1707,927 6,008 4 24 47 0,022 MKGCERKKR SVNPYLOGORLDNVVA KKSVPHFSD CAem 782 886,988 2 1008,688 0,0006 64 24 47 0,022 SKNPYLOGORLDNVVA KKSVPHFSDE CAem 782 886,988 2 1008,688 0,0006 64 24 40 0,0002 SKMKNPLGORDNPL CAem CgA 646,47358 2 1008,598 0,0001 65 24 40 0,0002 SKMKISFRAR AVGFROPCPOL RRGWRPSRE CgA 646,47358 2 1927,4207 0,0001 43 25 47 0,12 FRGFOPCPL WROPSREDLEACLPLO VROYFEEKKE CgA 666,6731 3 2095,065 0,0001 43 25 47 0,12 FRGFOPCPL GWRPSREDLEACLPLO VROYFEEKKE KROWRPSRE CgA 693,080 3 2094,0011 0,0010 44 47 0,002 KRETARRA <													
122 031 031 1700.8846 -0.0012 44 25 46 0.032 MKGGEERKKR SVNPYLOGORLDMVVA KKSVPHFSDE 7182 713.250 1500.0389 0.0007 51 28 47 0.022 MKGGEERKKR SVNPYLOGORLDMVVAK SVHFSDEDKDPE C-Rem CqA 513.250 196.00389 0.0007 69 32 45 0.0002 SIMKLSFRAR AVGR GPGPQ C-Rem CqA 644.7536 2 927.4927 -0.0001 40 37 46 0.19 MKGSFRAR AFGPGPQL RRGWRPSSRE CqA 664.6731 3 2196.0650 0.0004 42 24 0.0026 SIKKLSFRAR GRPGPQL RRGWRPSSRE <	After stin	nulation											
TB2 SB6.9805 2 T71,2217 0.0008 51 26 47 0.022 MKGGERLBNVA KKGGRLDNVA KKSUPHISDE TB2 T51.280 2 1500.638 0.006 64 2 40 0.0022 GRLDNVAKK SVPHISDEDKDPE C-#m CqA 552.2614 2 1161.5931 0.0001 65 29 45 0.0002 SMKLISFRAR AVGFR OF GP Q-Q RRGWRPSSRE CqA 464.7556 2 927.492 -0.001 43 25 47 0.12 FRGPGPQL RRGWRPSSRE SRGWRPSSREDSLEAGLPLQ VRGYPEEKKE CqA 666.6731 3 2.996.966 0.0014 43 25 47 0.12 FRGPGPQLRR GWRPSSREDSLEAGLPLQ VRGYPEEKKE CqA 699.3680 3 2994.011 0.010 59 18 4 0.0002 AKELTAFK LEGGEEEEINNDSSMLS FRARAYGFRG GRARAYGFRG GARAYGFRG GARAYGFRG GARAYGFRG GARAYGFRG GARAYGFRG GARAYGFRG GARAYGFRG GARAYGFRG GARAYGFRG GARAYGFRGPCPD GRARAYGFRG GARAYGFRGPCPQL	7B2	851 4489	2		1700 8846	-0.0012	44	25	46	0.083	MKGGERRKRR	SVNPYLOGORI DNVV	AKKSVPHESD
TP2 T91.3280 2 1500.8399 0.0008 64 22 40 0.0002 CRL DNV VAKK SV PHFSDEDKDPE C-lam CqA 525.2614 2 1104.509 -0.0007 69 32 46 0.0002 SMKLSFRAR AVGR OPG PQ Q RRGWRPSSRE CqA 649.3718 2 927.4927 -0.0011 40 37 46 0.0005 SMKLSFRAR VGR OPG PQ L RRGWRPSSRE CqA 669.4718 3 296.0650 0.0004 54 26 7 0.12 FRGOP OPL RGWRPSSREDSLEACLPLQ RGWRPSSREDSLEACLPLQ CqA 699.4688 3 296.0650 0.0004 54 26 47 0.002 AKE APAOOKK HSGFEDELSVLENQSSQAELKEAVEEPSSKD VME KREDSKEALKEE CqA 1302.4268 3 3965.763 -0.003 83 23 41 0.0002 KKE ITAKEK LEGOEEEEONROSSMLS FRA KREDSKEALKEE	7B2	886 9685	2		1771 9217	0.0008	51	26	47	0.022	MKGGERRKRR	SVNPYLOGORI DNVVA	KKSVPHESDE
CQA S25_2814 2 1048.5090 -0.0007 69 22 45 0.002 SIMILS FRARA A/GER GPGPO RRGW RPSSRE CqA 681.809 2 1161.5331 0.0016 65 29 45 0.0005 SSMILS FRARA OFROP GPQL RRGW RPSSRE CqA 666.8731 3 2 1996.9966 0.001 43 25 47 0.12 FRGEPORLIR GW RPSSREDSLEAGLPLQ VRGYPEEKKE CqA 669.5968 3 3905.7636 -0.0056 51 16 43 0.0022 ArcEntApock KREDSKALKAVEE KREDSKALKAVEER KREDSKALKAVEER KREDSKALKAVEER KREDSKALKAVEER KREDSKALKAVEERAVEERSKAVEERSKAVEERSKAVEERSKAVEERSKAVEERSKAVEERAVEERSKAV	7B2	751 3260	2		1500 6369	0.0006	64	22	40	0.00022	ORI DNVVAKK	SVPHESDEDKDPE	C-term
GA 581.8039 2 1161.5931 0.0001 65 29 45 0.0005 SSMKLSFRAR AYGFROPGPQL RRGWRPSSRE CgA 464.7386 2 927.4927 -0.001 40 37 46 0.19 MLSFRARAYY GFROPGPQL RRGWRPSSRE CgA 666.8731 3 2 1966.0650 13 2 196.0163 2006.0550 1001 43 24 7 0.012 FRGPCPOLR GWRPSSREDSLEAGLPLQ VROYPEEKKE CgA 699.6953 3 2006.0550 51 16 3 0.0002 AKERAHOCK HSGFEDELSEALLEYL CLAUCEESKALKE RRGWRPSSRE CgA 249.4303 3 2241.955 -0.003 83 24 41 3.0006 LKGCLTAKKR LEGOEEEEDNRDSSMKLS <fra< th=""> RAGVGFRGPC CgA 747.653 5 3768.7014 -0.0017 64 3.2 47 0.011 LKKELTAKKR LEGOEEEEDNRDSSMKLSFRA CKGWRPSSRE CgA 727.3265 3</fra<>	CaA	525,2614	2		1048,5090	-0.0007	69	32	45	0.0002	SSMKLSERAR	AY GER GP GP Q	L RRGW RPSSR
CgA 464.7536 2 927.4927 -0.001 40 37 46 0.19 MKLSFRARAY GFRGPGPQL RRGWRPSSRE CgA 666.6731 3 2 196.99966 0.0010 43 25 47 0.12 FRGPGPQLR GWRPSSREDSLEAGLPLQ VRYPEEKKE CgA 699.6568 3 3965.7638 -0.0056 51 16 43 0.0092 AKELTAEKR LEGOEEEEDNRDSSMKLS CMARSSQAELKEAVEEPSSKDVME RRAVGFRG CgA 699.6563 3 2044.9011 0.0016 51 8 41 0.0092 AKELTAEKR LEGOEEEEDNRDSSMKLS CMARSSQAELKEAVEEPSSKDVME RRAVGFRG CgA 744.3303 2 2449.1077 -0.0001 94 4 4.70E-07 LAKELTAEKR LEGOEEEEDNRDSSMKLSFRA CAGOERCPQL RRAVGFRGPQ CgA 774.365 3 2 2178.955 0.0017 64 2 47 0.0011 SKEWEDSKR WSKMDQLAKEL CAGOEEEDNRDSSMKLSFRA CAGOEEEDNRDSSMKLSFRA CAGOERCPQQL RAGGRGPQQL RAGGRGPQQL CgA 772.3265 3 2	CaA	581,8039	2		1161.5931	0.0001	65	29	45	0.00055	SSMKLSFRAR	AYGER GP GP QL	RRGWRPSSRE
CAL CORD CORD CAL CORD CAL CORD CAL CORD CAL CA	CaA	464 7536	2		927 4927	-0.0001	40	37	46	0.19	MKLSERARAY	GERGPGPO	RRGWRPSSRE
CgA 699.695 3 2096.0650 0.004 54 26 47 0.012 PROPSOLIRA PROPSOLIRA GW RPSSREDSLEAGLPLQV RGYPEEKKEE CgA 1302.926 3 2096.0650 0.005 51 16 43 0.0092 AKELARAHQOKK HSGFEDLSEVLENOSSOAELKEAVEEPSSKDVME KREDSKEAEK CgA 699.3080 3 2241.9695 -0.003 83 23 41 3.20E-06 CAKELTAEKR LEGOEEEEDNRDSSMKLSFRA CAGO RARAYGFRGP CgA 747.633 3 2 2178.9552 0.0013 37 47 0.51 LAKELTAEKR LEGOEEEEDNRDSSMKLSFRA RARAYGFRGPGPQL RRGWRPSSRE CgA 727.3255 3 2 2178.9552 0.0023 19 24 42 1.10E-08 VPOCLFRGGK SGELECEERNRDSSMKLSFRA CGA CGA CF3.3741 9.0017 64 32 47 0.0011 SKEWEDSK WSKMDQLAKEL X KRWSKMDQLAKEL X KRWSKMDQLAKEL X KRWSKMDQLAKEL X K	CaA	666 6731	3	2	1996 9966	0.0010	43	25	47	0.12	ERGPGPOIRE	GW RPSSREDSI FAGI PLO	VRGYPEEKKE
CA 1302.9266 3 3905.7636 -0.0056 51 16 43 0.0092 AKERAHQQKK HSGFEDELSEVLENQSSQAELKEAVEEPSSKDVME KREDSKEAKEK CgA 699.3080 3 2094.9011 0.0010 53 18 41 0.00092 AKELTAEKR LEGOEEEEDNRDSSMKLS FRARAYGFRG CgA 743.303 3 2449.1077 -0.0011 37 19 47 0.016 KRELTAEKR LEGOEEEEDNRDSSMKLSFRA RAGYGFRGPOP CgA 754.7653 5 3768.7914 -0.0011 37 19 47 0.016 KRELTAEKR LEGOEEEEDNRDSSMKLSFRARAYGFRGPGPQL RRGWRPSSRE CgA 772.73265 3 2 1347.6856 -0.0017 64 32 47 0.0016 SKEWEDSKR WSKMDQLAKEL TAKKLEGOE KRWSKMDQLA KREGOEEEE KREGOEEEEE KREGOEEEEE KREGOEEEEE KREGOEEEEE <t< td=""><td>CaA</td><td>699 6958</td><td>3</td><td>-</td><td>2096.0650</td><td>0.0004</td><td>54</td><td>26</td><td>47</td><td>0.012</td><td>FRGPGPGIRR</td><td>GW RPSSREDSLEAGL PLOV</td><td>RGYPEEKKEE</td></t<>	CaA	699 6958	3	-	2096.0650	0.0004	54	26	47	0.012	FRGPGPGIRR	GW RPSSREDSLEAGL PLOV	RGYPEEKKEE
CgA CGA <thca< th=""> <thcga< th=""> <thcga< th=""></thcga<></thcga<></thca<>	CaA	1302 9266	3		3905 7636	-0.0056	51	16	43	0.012		HSGEEDELSEVIENOSSOAELKEAVEEPSSKDVME	KREDSKEAEK
CgA 748.3303 3 2494.9511 6.0010 35 10 41 0.0002 2448.2174.KTK LEGGLELEDINEDSMILSJ RARAYGERGP RARAYGERGP CgA 748.3303 2469.1077 -0.0011 37 44 3.204.0 AKELTAKK LEGGLEEEDNNDSSMILSFRA RAYGERGPGPL RARAYGERGPGPL RARAYGERG	CaA	600.3080	3		2004 0011	0.0000	50	19	41	0.0002			ERABAYCERC
CgA 62.4.0432 2 14.0.03 6.0.01 64.0 16.4.02 Late Content of the Content of	CaA	748 3303	3		2241 9695	-0.0003	83	23	41	3 20 E-06			RARAYGERGP
CgA CgA 754.765 5 3768.7914 -0.001 37 19 47 10 40.01 4.102.71 LEGGELEEDARDK LEGGELEEDARDKARY EEGGEEGENRDSSMKLSFRARAYGFRGPGPQL RRGWRPSSRE CgA 727.325 3 2 2178.9552 0.0023 19 42 1.102-08 VPGQLFRGGK SGELCCEEERLSKEWEDS KRWSKMDQLAKEL TAEKRLEGOE CgA 674.8492 2 1519.7704 0.0015 54 44 0.0006 LSKEWEDSKR WSKMDQLAKEL TAEKRLEGOE ERGUEEEE CgA 760.8932 2 1519.7704 0.0015 54 44 0.0006 LSKEWEDSKR WSKMDQLAKEL TAEKRLEGOEE ERGUEEEEE CgA 254.145 2 3 164.8130 0.0014 92 74 6 SKEWEDSKR WSKMDQLAKEL TAEKRLEGOEE CgA 456.2849 2 1090.5560 -0.0007 27 16 43 3.5 SKEWEDSKR WSKMDQLAKEL TAEKRLEGOEE RGWRPSSRE CgB CgB 624.7640	CaA	824 0432	3		2469 1077	-0.0000	04	18	43	4 70 5-07			PAVGERGROP
CgA 727.3265 3 2 2178.9552 0.0023 10 0.01 MELDALEMANN Cold Leibnack Meddation Med	CaA	754 7653	5		3768 7014	-0.0013	37	10	40	0.51			PRGWPPSSPE
GA 674.8492 2 1347.6856 -0.007 64 32 47 0.0011 LSKEW EDSKR WSKMDQLAKEL TAEKRLEGQE CgA 725.374 2 1448.733 0.0003 63 26 44 0.0006 LSKEW EDSKR WSKMDQLAKEL T AEKRLEGQEE CgA 765.874 2 3 1648.813 0.0014 92 27 46 1.502-06 LSKEW EDSKR WSKMDQLAKEL TA KRLEGQEEE CgA 762.874 2 3 1648.813 0.0014 92 27 46 1.502-06 LSKEW EDSKR WSKMDQLAKEL TA KRLEGQEEE CgA 546.2449 2 1990.5560 -0.0007 27 16 44 3.4 SMKLSFRARA YGFROPOPQL RROWRPSSRE CgB 642.7640 2 1247.5128 0.0006 56 29 40 0.0013 HHRGRGGEPR AYFMSDTREE RROWRPSSRE KREIGEGHHR RASEEPPYG CgB 689.3619 3 2065.0327 0.0010 78 22 4 4.002-45 VLYKISRKOVK DKETTENENTKFEVRLL <	CaA	727 3265	3	2	2178 9552	0.0023	109	24	42	1 10E-08	VPOGLERGGK	SGELEDEFERI SKEWEDS	KRWSKMDOLA
OrgA Street OrgA Street OrgA OrgA OrgA OrgA Street OrgA Street OrgA	CaA	674 8492	2	-	1347 6856	-0.0017	64	32	47	0.0011	I SKEWEDSKR	WSKMDOLAKEL	TAEKPLEGOE
CgA 7253/47 2 1519.7704 0.0015 54 26 40 0.0030 LSKEWEDSKR WSKMOQLAKELTA ERRLEGGEEE CgA 625.4125 2 3 1648.4130 0.0014 52 27 46 1.50E-06 LSKEWEDSKR WSKMOQLAKELTA ERRLEGGEEE CgA 654.2849 2 190.560 -0.0007 27 16 44 3.4 SMKLSFRARA YSFROPCQL REGOTEEE CgB 624.7640 2 1247.5128 0.0006 56 29 40 0.0013 LHKREGGEPR AYFMSDTREE KRECOGEEEE KRECOGEEHE KRECOGEEHE <td>CaA</td> <td>725 3741</td> <td>2</td> <td></td> <td>14/8 7333</td> <td>0.0003</td> <td>63</td> <td>26</td> <td>44</td> <td>aanno.0</td> <td>I SKEWEDSKR</td> <td>WSKMDQLAKELT</td> <td>AEKRIEGOEE</td>	CaA	725 3741	2		14/8 7333	0.0003	63	26	44	aanno.0	I SKEWEDSKR	WSKMDQLAKELT	AEKRIEGOEE
CgA 625A145 2 1515,174 0.0015 34 24 0 0.0055 EXEWEDS/NC WSM/DGLAKELTAE KRLEGGEEEE CgA 625A145 2 3 1648,130 0.0014 92 7 46 1.5056 LSKEWEDS/NC WSM/DGLAKELTAE KRLEGGEEEE CgA 625A145 2 1905,550 -0.0007 27 16 44 3.4 SMKULSFRARA YGFROPOPQL RROWRPSSRE CgB 624,7640 2 1247.5128 0.0006 56 29 40 0.0013 HHRGRGGEPR AYFMSDTREE RASEEPFYG RASEEPFYG CgB 689,3519 3 2065,0327 0.001 78 22 4 4.00-49 VIXASLGEKR DHETTENENTKEVRLL RDAJASEAH CgB 665,9850 3 1994,931 -0.0004 87 21 46 3.0046 FLGEGHHR KRLEGGEHER KRLEGGEHER KRLEGGELEE RRHPGGAWKE KREGEGERAY/FMSDTREE KREGEGERAY/FMSDTREE KREDGEGEHR KRRLEGACEEGHR	CaA	760 9022	2		1510 7704	0.0005	54	20	46	0.00000			
CgA 54/284 2 5 1048/31/30 2 2 104/31/30 10/31/30 1	CgA	925 4145	2	2	16/9 91 20	0.0013	0.2	20	40	1 50 5 0.0			KRIEGOEEEE
CgB Cg2 195.0500 -0.007 27 10 44 3.4.3 SML5FRARA TOFROFORUL RROKE RROKE CgB 624.7640 2 124.75128 0.0006 56 24 40.0013 HRORGOFRARA TOFROFORUL KRRLDEGHHR KRRLDEGHR CgB 448.6857 2 895.5573 -0.0005 15 14 41 20 VSMASLGEKR DHHSTHY RASEEPEYG CgB 689.3571 3 2065.0327 0.0010 78 22 47 4.60E-05 VLTSRKOVK DKETTENENTKFEVRLL RDPAASEAH RDPAASEAH CgB 699.6091 3 179.6006 -0.0004 21 74 4.60E-05 VLTSRKOVK DKETTENENTKFEVRLL RDPAASEAH RDPAASEAH RDPAASEAH RDPAASEAH RCPACHRVOENOMD KARRHPOGAW RRHPOGAWKE CgB 665.9865 3 1994.9381 -0.0004 87 21 46 3.70E-06 FMSDTREE FLGEGHHRVOENOMD KARRHPOGAWKE KREGEGHRVOENOMDKA RRHPOGAWKE	CaA	546 2040	2	3	1000 5560	0.0014	32	16	40	1.301-00	SMKL SEDADA	VCERCROROL	RECWERSE
CgB CgB <td>CaP</td> <td>624 7649</td> <td>2</td> <td></td> <td>1090.0000</td> <td>-0.0007</td> <td>21</td> <td>20</td> <td>44</td> <td>3.4</td> <td>UUDODOCODD</td> <td></td> <td>KREIGEGUUD</td>	CaP	624 7649	2		1090.0000	-0.0007	21	20	44	3.4	UUDODOCODD		KREIGEGUUD
Cgb 669.359 3 206.000 7.0005 10 14 41 2000000000000000000000000000000000000	CaB	024./040	2		1241.0120	0.0006	50	29	4U 44	0.0013	MARGER PR		DASEEEDEVC
CgB 665,985 3 195,8060 -0.004 21 4 0.2024 FMSDTREEKR FLGEGHRVQENOMD MARIFEVENLL RRHPQGAW CgB 665,9855 3 1994,9381 -0.004 21 46 3.702-06 FMSDTREEKR FLGEGHRVQENOMD KARRHPQGAW RRHPQGAW CgB 665,9865 3 1994,9381 -0.004 87 21 46 3.702-06 FMSDTREEKR FLGEGHRVQENOMD KARRHPQGAW RRHPQGAW CgB 665,2990 3 1956,8748 0.0004 58 19 43 0.0019 EPGEGRHHR GRG GEPRAYFMSDTREE KREICEGHRK KREICEGHRK RCBOMMSMDNEL RRDNMNDNEL CgB 824,0616 3 2469,1641 -0.0013 36 32 48 8.102-06 YSSHITAEKR RIGELRNPYVPDLQWKSSHFE RRDNMNDNEL CgB 569,8506 2 1137,6870 -0.003 35 32 41 0.24 EDVNW GYEKR NLARVPKLDL KARVPKLDL KRQYDRVAQL	CaB	440.000/	2		095.3573	-0.0005	15	14	41	4 60 5 0 5	VSWASLGERR		RASEEPEIG
CgB 665.9865 3 195.0004 87 21 47 3.0015 FLOE GHIRK VELENAND RRHPOGAWKE CgB 665.9865 3 195.431 -0.004 87 21 46 3.0016 FLOE GHIRK VELENAND RRHPOGAWKE CgB 653.9805 3 1956.8748 0.0004 87 21 46 3.0016 EPG KGRH HR CRG GEPRAY FMSDTREE KRELGEGHIRK RCLEGEGHIRK CgB 689.3477 4 2753.3602 0.0013 86 23 48 8.10E-06 FVSHTAEKR KRLGELFNPYYDPLQWKSSHFE RRDNMNDNFL CgB 624.0616 3 2469.1641 -0.0012 35 32 41 0.24 EDVNW GYEKR NLARVPKLDL KRQYDRVAQL KRQYDRVAQL	CaB	009.3019	3		2005.032/	0.0010	18	17	47	4.000-05	EMEDTREEKP		KARRHROCAN
Cgb 000.3005 3 1994.9501 -0.0004 87 21 46 3.702-105 DT REERK FLGE GHHRV DENDMIKA RRHPGGAWKE RRHPGGAWKE CgB 653.2990 3 1956.8748 0.0004 58 19 43 0.0019 LEPGKGRH R GRG GEPRAYEMSDTREE KRL9EGHHRVDENDURA KRL9EGHHRVDENDURA CgB 689.3477 4 27.53.602 0.0013 86 9 43 0.0019 LEPGKGRH R GRG GEPRAYEMSDTREE RRDNMNDNFL KRQYDRVAQL	CyB C-D	099.0091	3		1/ 95.6000	-0.0004	21	17	44	9.8	FMODTREEKR		RARRIPQGAW
Cgb 659.2479 3 1950.6740 0.0004 58 19 43 0.0019 Createst extention CREATEST extended KRPLEGERHAR	CgB C-B	000.9865	3		1994.9381	-0.0004	87	∠1 40	40	3.70E-06	HISDIREEKR		KRHPQGAWKE
Cgb 5824.0616 2 48.0616 24.081	CgB C-B	053.2990	3		1956.8748	0.0004	58	19	43	0.0019	LEPGKGRHHR		REFLGEGHER
Cgb C240010 3 24001041 -0.0012 33 19 40 0.031555HTAEKK C5ELFMEYTDPLQWKSSHFE RKDNMNDNE CgB 569.8506 2 1137.6870 -0.0003 35 32 41 0.24 EDVNW GYEKR NLARVPKLDL KRQYDRVAQL	CaB	009.34//	4		21 00.3002	0.0013	86	23	40 40	0. IUE-06	VESHITAEKR		REDNMNDNEL
CGB 009/00/0 Z 113/.00/U -0.00/U 3 3 32 41 0.24 EDVNWGYEKK NLAKVYKLUL KROYDRVAOL	Cyb C-D	024.0010	0		2409.1041	-0.0012	35	19	40	0.05	TO THE REAL		
	CGR	269.8206	Z		1137.6870	-0.0003	35	32	41	0.24	EDVNWGYEKR	NLARVPALUL	KRQYDRVAQL

CgB	731.1431	5		3650.6808	-0.0015	50	4	5	0.016 EDVNW GYEKR	NYPSLELDKMAHGYGEESEEERGLEPGKGRHH	RGRGGEPRAY
CgB	671.7838	2		1341.5513	0.0016	22	16 4	1	4.4 EYNYDWWEKK	PFSEDVNWGYE	KRNLARVPKL
CaB	872.9364	2		1743.8580	0.0003	61	32 4	6	0.0016 ARVPKLDLKR	<pre><qydrvaqldqllhy< pre=""></qydrvaqldqllhy<></pre>	RKKSAEFPDF
CgB	587.9688	3		1760.8846	0.0000	64	21 4	7	0.001 ARVPKLDLKR	QYDRVAQLDQLLHY	RKKSAEFPDF
CgB	1068.4681	3		3202.3844	-0.0019	100	21 4	0 5	80E-08 HHHGRSRPDR	SSQGGSLPSEEKGHPQEESEESNVSMASLGE	KRDHHSTHYR
CGRP	1186.6031	5	6 5	5927.9850 6056.0436	-0.0059	136 156	24 5	01. 01	50E-10 Signal 60E-12 Signal	APFRSALESSPADPATI SE DEARLILIAL VQD YV QMKASELE QE QERE GSRIIA APERSALESSPADPATI SE DEARLI I AAL VQD YV QMKASELE QE QERE GSRIIAO	QKRACDIATC KRACDIATCV
CGRP	734.3766	3	0	2200.1083	-0.0004	52	20 4	6	0.014 AALVQDYVQM	KASELEQEQEREGSRIAQ	KRACDTATCV
CGRP	877.8294	3		2630.4657	0.0006	118	4	3 2	10E-09 CDTATCVTHR	LAGLLSRSGGVVKNNFVPTNVGSKAF-NH2	GRRRRDLQA
CGRP	797.4484	3		2389.3230	0.0002	81	4	4 1. -	20E-05 ATCVTHRLAG	LLSRSGGVVKNNFVPTNVGSKAF-NH2	GRRRRDLQA
CGRP	778.0572	3		2203.0902	0.0000	55 100	34 4	73			KRACDIATC
CGRP	788.4104	2		1574.8053	0.0010	38	21 4	7	0.43 THRLAGLLSR	SGGVVKNNFVPTNVGS	KAFGRRRR
CGRP	961.0179	2		1920.0218	-0.0005	96	27 4	65.	90E-07 THRLAGLLSR	SGGVVKNNFVPTNVGSKAF-NH2	GRRRRDLQA
CGRP	509.7875	2		1017.5607	-0.0003	38	22 4	6 7	0.3 RSGGWKNNF	VPTNVGSKAF-NH2	GRRRRDLQA
CGRP	649.3277	4		2593.2805	0.0010	71	17 4	7 7 (0.00025 RELEAREVOD	YVOMKASELEGEGEREGSRING	OKRACDIATC
CGRP	908.1203	3	4	2721.3391	-0.0001	120	33 4	7 3	30E-09 RLLLAALVQD	YVQMKASELEQEQEREGSRIIAQ	KRACDTATCV
СТ	1037.1800	6	5	6217.0396	-0.0033	143	23 4	9 2	50E-11 Signal	APFRSALESSPADPATLSEDEARLLLAALVQDYVQMKASELEQEQEREGSSLDSPR S	KRCGNLSTCM
CT	1247.6136	5		6233.0345	-0.0029	58	4	9	0.0077 Signal	APFRSALESSPADPATLSEDEARLILAALVQDYVQMKASELEQEQEREGSSLDSPRS	KRCGNLSTCM
CT	610.7458	2		1219.4775	-0.0005	54	21 3	6 (0.00099 GVGAPGKKR	DMSSDLERDH	RPHVSMPQNA
СТ	532.2388	3		1593.6954	-0.0009	29	16 4	2	1.1 IGVGAPGKKR	DMSSDLERDHRPH	VSMPQNAN
CT	756.3361	3		2265.9855	0.0008	40	16 4	1	0.079 GVGAPGKKR	DMSSDLERDHRPHVS <u>M</u> PQN	AN
CT	780.0152	3		2337.0226	0.0013	24 49	16 4	2	0.011 IGVGAPGKKR	DMSSDLERDHRPHVSMPQNA	N
СТ	774.6831	3		2321.0277	-0.0002	65	20 4	3 (0.00033 IGVGAPGKKR	DMSSDLERDHRPHVSMPQNA	N
CT	812.6975	3	2,4	2435.0706	0.0000	65	21 4	2 (0.00031 IGVGAPGKKR	DMSSDLERDHRPHVSMPQNAN	C-term
CT	818.0293 721.3836	3		2451.0655	0.0005	59 53	1/ 4 29 4	1 5	0.0009 GVGAPGKKR	DMSSDLERDHRPHVS <u>M</u> PQNAN EHTEPOTAIGVGAP-NH2	C-term CKKRDMSSDI
СТ	831,7223	3	4	2492.1449	0.0002	134	27 4	56.	40E-11 LAALVQDYVQ	MKASELEQEQEREGSSLDSPRS	KRCGNLSTCM
СТ	490.7348	2		979.4545	0.0005	41	32 4	3	0.09 MSSDLERDHR	PHVSMPQNA	N
CT	547.7556	2		1093.4975	-0.0007	37	23 4	3	0.26 MSSDLERDHR	PHVSMPQNAN	C-term
CT/CGRP	901.7850 674.3307	3		2882.3352	-0.0003	134	22 4	57. 4	0.14 Signal	APERSALESSED	PATLSEDEAR
CT/CGRP	881.7808	3		2642.3187	0.0020	106	23 4	88	.60E-08 Signal	APFRSALESSPADPATLSEDEARLL	LAALVQDYVQ
CT/CGRP	919.4757	3		2755.4028	0.0025	28	22 4	8	5.1 Signal	APFRSALESSPADPATLSEDEARLLL	AALVQDYVQM
CT/CGRP	1118.5786	3		3352.7150	-0.0010	61	23 4	8	0.003 Signal	APFRSALESSPADPATLSEDEARLLLAALVQD	YVQMKASELE
GRP	560.3060	2		1110.5972	-0.0002	39 28	21 4	0 5	2.8 GTVLTKMYPR	GNHWAVGHIM-NH2	GKKSTGESSS
GRP	670.8837	2		1339.7534	-0.0004	62	28 4	- 1 (0.00039 Signal	VPLPAGGGTVLTKM	YPRGNHWAVG
GRP	752.4157	2		1502.8167	0.0001	64	22 4	3	0.0004 Signal	VPLPAGGGT VLTKMY	PRGNHW AVGH
GRP	800.9420	2	3	1599.8695	-0.0001	50	21 4	3	0.011 Signal	VPLPAGGGTVLTKMYP	RGNHWAVGHL
GRP	808.9396	2	3	1015.8044	-0.0003	63 139	26 4	4 (3 1.	.40E-11 Signal	VPLPAGGGTVLTKMYP	GNHWAVGHL
GRP	591.6626	3	, e	1771.9655	0.0003	54	23 4	5	0.007 Signal	VPLPAGGGTVLTK <u>M</u> YPR	GNHWAVGHLM
KRT8	479.9398	3		1436.7987	-0.0010	60	27 4	4	0.0014 M	Ac-SIRVTQKSYKVS	TSGPRAFSSR
KRT8	513.6225	3		1537.8464	-0.0008	39	18 4	4	0.17 M	AC-SIRVTQKSYKVST	SGPRAFSSRS
NPW	541.8009 413.5702	2		1237 6891	-0.0007	28	17 4	5 1	1.2 VOELWEIRRR	SSOAGIPVRAP	SPRAPEPAL
PACAP	774.3423	2		1546.6688	0.0012	31	18 4	2	0.75 GDD AEPLSKR	HSD GIFTDSY SRY	RKQMAVKKYL
PC2	715,7713	5		3573.8229	-0.0030	88	21 4	8 5	50E-06 Signal	ERPVFTNHFLVELHKGGEDKARQVAAEHGFGV	RKLPFAEGLY
PC2	813.5659 496.5950	7		5687.9079	0.0027	44	24 4	0	0.19 Signal	ERPVFTNHFLVELHKGGEDKARQVAAEHGFGVRKLPFAEGLYHFYHNGLA	RVKMALOOFG
PC2 PC2	490.5950 633.3134	2		1264 6122	0.0009	69	24 4	0 5 (0.018 NGLANARRAR	VKMALQOEGED	RKKRGYRDIN
PC2	641.3111	2		1280.6071	0.0006	72	27 4	4 9.	20E-05 HKQQLERDPR	VK <u>M</u> ALQQEGFD	RKKRGYRDIN
PENK	693.8411	2		1385.6674	0.0002	62	28 4	6	0.0013 YGGFMRGLKR	SPQLEDEAKELQ	KRYGGFMRRV
ProSAAS	743.6491	4		2970.5635	0.0037	53	21 4	7	0.013 ETGAPRRFRR		RARAEAQEAE
Sall	1088,2673	6 4		4982.4661	-0.0039	37 112	4	9 6 1.	50E-08 EINSNOVKR	VPG OG SSEDD LØ EE ØIE ØAIKEHL NO GSSØET DKLAPVS	KREPVGPPKN
SgIII	643.3237	2		1284.6310	0.0019	49	29 4	6	0.029 GSQDKSLHNR	ELSAERPLNEQ	AEAEEDKIK
SgIII	699.8649	2		1397.7150	0.0002	41	26 4	6	0.18 GSQDKSLHNR	ELSAERPLNEQ	AEAEEDKIKK
SgIII	735.3837	2		1468.7521	0.0007	44	41 4	4 6	0.066 GSQDKSLHNR		EEDKIKKTYP
Sall	799.9048 899.9447	2		1797.8744	0.0005	49 67	22 4	о 6 (0.00047 GSQDKSLHNR	ELSAERPINEGIAEAE	EDKIKKTYPP
SgIII	964.4659	2		1926.9170	0.0002	99	32 4	5 2	30E-07 GSQDKSLHNR	ELSAERPLNEQIAEAEE	DKIKKTYPPE
SgIII	1021.9788	2		2041.9439	-0.0009	98	21 4	5 2	80E-07 GSQDKSLHNR		KIKKTYPPEN
Sall	142.5676	2	3	2283.1230	-0.0023	95	32 4	78. 5	50E-07 GSQDKSLHNR	ELSAERPLNEQIAEAEEDKI FPKPGGSOD	KKTYPPENKP
SgIII	630.8329	2		1259.6510	0.0002	27	25 4	5	3.4 Signal	FPKPGGSQDKSL	HNRELSAERP
SgIII	504.5913	3	2	1510.7528	-0.0007	48	22 4	5	0.025 Signal	FPKPGGSQDKSLHN	RELSAERPLN
SgIII	862.6868	4		3446.7178	0.0004	22	19 4	8	24 Signal	FPKPGGSQDKSLHNRELSAERPLNEQIAEAE	EDKIKKTYPP
og∎ Sa∭	923./038 787.4007	4 5	6	3090.7873 3931.9663	-0.0013 0.0008	50 59	19 4	а 9	0.033 Signal 0.0055 Signal	FFREGGSQUKSLHNRELSAERFLNEQIAEAEED FPKPGGSQDKSLHNRELSAERPLNEQIAEAEEDKI	KKTYPPEN
SgIII	661.1581	6	0	3960.9050	0.0000	71	4	8 (0.00027 EWLKKHDKK	GNKEDYDLSKMRDFINKQADAYVEKGILDKEEAE	AIKRIYSSL
SgIII	830.0125	5	6	4145.0262	-0.0002	95	23 4	8 1.	40E-06 EWLKKHDKK	GNKEDYDLSKMRDFINKQADAYVEKGILDKEEAEAI	KRIYSSL
SgIII	999.5105	5	6	4992.5178	-0.0018	177	5	0 1.	10E-14 IEWLKKHDKK	GNKEDYDLSKMRDFINKQADAYVEKGILDKEEAEAIKRIYSSL	C-term
Sall	591.8170 866.9154	2		1731.6193	-0.0001	47 52	23 4	4 5	0.026 TEAYLEAIRK	NEWLKKHD SSPLDNKLNVEDVDST	KNRKLIDDYD
SST	480.2160	2		958.4178	0.0004	31	26 4	2	0.82 QDEMRLELQR	SANSNPAMAP	RERKAGCKNF
SST	622.7879	2		1243.5615	-0.0002	74	20 4	2 3.	30E-05 QDEMRLELQR	SANSNPAMAPRE	RKAGCKNFFW
TMSB10	987.7114	5	6,7	4933.5229	-0.0022	131	4	93.	.80E-10 M	Ac-ADKPDMGEIASFDKAKLKKTETQEKNTLPTKETIEQEKRSEIS	C-term
TMSB4X	1241.1269	4	5, 6.7	4960.4862	-0.0021	47 100	4	95.	.10E-07 M	AC-SDKPDMAEIEKFDKSKLKKTETQEKNPLPSKETIEQEKQAGES	C-term
TMSB4X	996.3027	5	, . . .	4976.4811	-0.0041	93	4	9 2	20E-06 M	Ac-SDKPDMAEIEKFDKSKLKKTETQEKNPLPSKETIEQEKQAGES	C-term
VGF	892.9472	4		3567.7594	0.0003	47	23 4	8	0.074 Signal	APPGRPEAQPPPLSSEHKEPVAGDAVPGPKDGSAPE	VRGARNSEPQ
VGF	1223.2823	3	4,5	3666.8278	-0.0027	72	19 -	-8 (0	0.00024 Signal		
VGF	988.7539	ა 4	5.6	3950.9875	-0.0014	∠3 119	23 4	95	40E 09 Signal	APPGRPEAQPPPLSSEHKEPVAGDAVFORDUGAPEVKG	RNSEPQDEGE
VGF	779.8765	2	, -	1557.7383	0.0002	53	23 4	5	0.0078 YPGREAQARR	AQEEAEAEERRLQ	EQEELENYIE
VGF	927.4661	4	5,6	3705.8346	0.0009	112	4	8 2	40E-08 YPGREAQARR	AQEEAEAEERRLQEQEELENYIEH VLLRRP	C-term
VGF	542.5338	4	n	2166.1069	-0.0010	39 104	16 4	6 1 4	0.3 EAEAEERRLQ	EQEELENYIEHVLLRRP	C-term
VGF	507.0214	4	∠ 3	2023.9877	-0.0011	44	20 2	6	0.098 LQPPSALRRR	HYHHALPPSRHYPGREA	QARRAQEEAE
VGF	718.3561	3	4	2152.0463	0.0002	56	20 4	6	0.005 LQPPSALRRR	HYHHALPPSRHYPGREAQ	ARRAQEEAEA
VGF	742.0357	3	4	2223.0834	0.0018	63	21 4	7	0.0014 LQPPSALRRR	HYHHALPPSRHYPGREAQA	RRAQEEAEAE
vG⊢	537.6075	3		1609.8014	-0.0006	19	15 4	-D	28 LOPPSALRRR	HTHHALPPORHTP-NH2	GREAQARRAQ

TABLE I—continued

NOF	540 5570		0	0170 00 11	0.0045		~				DODODDDDAD
VGF	543.5572	4	3	2170.2011	-0.0015	69	21	44	0.00019 EEVEEKRKR	KKNAPPEPVPPPRAAPAPIHV	RSPQPPPPAP
VGF	602.8200	4		2407.2495	0.0015	68	19	47	0.00043 QEEAEAEERR	LQEQEELENYIEHVLLRRP	C-term
VGF	909.6714	5	6	4543.3210	-0.0002	145		49	1.50E-11 PPAPSQFQAR	MPDSGPLPETHKFGEGVSSPKTHLGEALAPLSKAYQGVAAPFPK	ARRPESALLG
VGF	923.8788	5	6	4614.3581	-0.0002	118	22	49	7.60E-09 PPAPSQFQAR	MPDSGPLPETHKFGEGVSSPKTHLGEALAPLSKAYQGVAAPFPKA	RRPESALLGG
VGF	585.8179	2		1169.6193	0.0019	43	23	46	0.11 EVEEKRKRKK	NAPPEPVPPR	AAPAPTHVRS
VGF	839.9493	2		1677.8838	0.0002	83	28	46	1.10E-05 EVEEKRKRKK	NAPPEPVPPRAAPAPT	HVRSPQPPPP
VGF	958.0125	2	3	1914.0112	-0.0008	89	22	46	3.30E-06 EVEEKRKRKK	NAPPEPVPPRAAPAPTHV	RSPQPPPPAP
VGF	958.9349	2		1915.8548	0.0005	35	18	43	0.35 GSAPEVRGAR	NSEPQDEGELFQGVDPR	ALAAVLLQAL
VGF	828.8768	2		1655.7387	0.0002	77	23	43	2 10E-05 RDFSPSSAKR	<qqetaaaetetrtht< td=""><td>LTRVN LESPG</td></qqetaaaetetrtht<>	LTRVN LESPG
VGF	747.3683	3		2239.0829	0.0001	48	17	47	0.043 RDFSPSSAKR	<qqetaaaetetrthtltrvn< td=""><td>LESPGPERVW</td></qqetaaaetetrthtltrvn<>	LESPGPERVW
VGF	848.4226	4	3	3389.6600	0.0011	93	19	48	1.70E-06 RDFSPSSAKR	<qqetaaaetetrthtltrvnlespgpervw< td=""><td>RASW GEFQAR</td></qqetaaaetetrthtltrvnlespgpervw<>	RASW GEFQAR
VGF	629,9729	3		1886.8970	-0.0002	23	14	46	9.3 RDFSPSSAKR	QQETAAAETETRTHTLT	RVNLESPGPE
VGF	989.4860	3		2965.4377	-0.0017	72	17	47	0.00021 RDFSPSSAKR	QQETAAAETETRTHTLTRVNLESPGPE	RVWRASW GEF
VGF	852.6789	4	3,5	3406.6865	-0.0002	118	22	48	5.70E-09 RDFSPSSAKR	QQETAAAETETRTHTLTRVNLESPGPERVW	RASW GEFQAR
VGF	572,2870	3		1713.8394	-0.0002	54	25	46	0.009 HYPGREAQAR	RAQEEAEAERRLQ	EQEELENYIE
VGF	773.3947	5		3861.9357	0.0013	56		49	0.0099 HYPGREAQAR	RAQEEAEAEERRLQEQEELENYIEHVLLRRP	C-term
VGF	641.8454	4		2563.3506	0.0018	59	22	46	0.0032 AQEEAEAEER	RLQEQEELENYIEHVLLRRP	C-term
VGF	442.6910	2		883.3672	0.0003	32	25	41	0.41 DGEAGAED KR	SQEETPGH	RRKEAEGTEE
VGF	727.3609	2		1452.7069	0.0003	17	16	44	32 DGEAGAED KR	SQEETPGHRRKE	AEGTEEGGEE
VGF	698.0365	3		2091.0861	0.0016	40	17	46	0.24 ETAAAETETR	THTLTRVNLESPGPERVW	RASW GEFQAR
VGF	691.8575	2		1381.6990	0.0014	35	24	46	0.88 TETRTHTLTR	VNLESPGPERVW	RASW GEFQAR
VGF	577.6458	3		1729.9151	0.0003	37	21	45	0.44 RASW GEFQAR	VPERAPLPPPAPSQFQ	ARMPDSGPLP

TABLE I—continued



FIG. 3. Representative base peak chromatograms of the secretopeptidome from unstimulated (*top*) and stimulated (*bottom*) cells. Samples without gel filtration chromatography were analyzed. Major processing products of CT and CGRP precursors are illustrated along with *arrows* pointing at their peaks in the chromatogram. The base peaks marked with *asterisks* at 20.85 min (unstimulated) and 21.50 min (stimulated) are intact ubiquitin. *SP*, signal peptide; *N-term pro.*, N-terminal propeptide; *Kat.*, katacalcin. Base peaks at 8.75 and 15.03 min in the *top panel* were unrelated to peptide signals. *RT*, retention time; *NL*, normalized ion intensity.

With regard to the SST precursor, the 1243.56-Da peptide corresponds to the first 12 amino acids of SST-28. The dibasic sites RK (position 78–79) located downstream of this 12-residue peptide (Table II) is the known cleavage site for SST-14 (AGCKNFFWKTFTSC) (20). The single arginine (position 65) flanking the 1243.56-Da peptide and SST-28 is also an established processing site (20). At position -4 relative to this scissible bond (referred to as the P4 position), a single arginine (position 62) exists and forms a consensus

PC cleavage site. As for the GRP precursor, the 1599.87-Da peptide is C-terminally flanked by an atypical single arginine, which is followed by intact neuromedin C (21). This arginine is known as an established processing site, although responsible enzymes remain to be identified. Thus, a majority of the cleavage sites of peptide hormone precursors were consistent with the previously identified processing sites. Except for precursor C termini, all the peptides derived from granin-like precursors (SgII, SgIII, and VGF) retained informative cleavage sites defined in this study. The 2677.41-Da VGF-derived amidated peptide was recently identified and designated NERP-1 (16).

Integrity of the Secretopeptidome Demonstrated by an In-depth Analysis-The secretopeptidome was separated into four fractions using gel filtration HPLC to perform an in-depth analysis. This analysis contributed to a substantial increase in sequenced peptides, and thus we were able to identify a total of 400 peptides from 23 precursors (Fig. 4 and the supplemental table). Some peptides arose from precursors not identified by the one-dimensional analysis; these included peptide hormone precursors neuromedin U and ghrelin, processing enzymes PC1 and peptidyl-glycine α -amidating monooxygenase, and the calcium-binding protein calnuc. The identification of PC1- and peptidyl-glycine a-amidating monooxygenase-derived peptides confirmed the integrity of this secretopeptidome as they are enzymes involved in the RSP proteolytic processing. Four calnucderived peptides had typical cleavage sites suggestive of the PC function (supplemental table). Recently calnuc was identified in a soluble fraction of bovine adrenal secretory granules (13). Altogether it is likely that nearly 99% of sequenced peptides were released upon exocytosis, which again demonstrates that this secretopeptidome is extremely rich in peptides stored in secretory granules.

We examined a total of 400 sequenced peptides to see whether they meet the criteria for the PC consensus sites (supplemental table). PC consensus sites were found in 299 peptides, and signal sequence cleavage sites were found in 43

Table II

Identity of the major peptides released on exocytosis (base peak intensities beyond 2e+06 in the Fig. 3 base peak chromatogram) Values in brackets represent those obtained by peptides after reductive alkylation. For the ubiquitin MSMS spectrum, see supplemental Fig. 2. Note that the MSMS spectra of the 1278.67- and 5687.91-Da peptides (supplemental Fig. 3) did not meet the homology threshold criteria but were considered identified as described in the text. Grayscale boxes are defined in Table I legend. Oxidized methionine residues are underlined in column 10. TMSB4X, thymosin β -4 X-linked; TMSB10, thymosin β -10; RT, retention time; obsd, observed; Mr(Calc), theoretical monoisotopic molecular mass (Da) based on the peptide sequence; Expect., Expectation; N-term, the N-terminal flanking 10 amino acids; C-term, the C-terminal flanking 10 amino acids.

RT (min)	m/z (obsd.)	z	Mr(Calc)	Mass error	Base peak	Score	Expect.	N-term	Sequence	C-term	Pre-	Validated
(11111)	(0000)			(ppm)	intensity		value				001301	by
8.85	622.7882	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	FPKPGGSQDKSLHN	RELSAERPLN	SgIII	
12.55	613.7739	4	2451.0655	0.40	2.78E+06	59	0.0009	GVGAPGKKR	DMSSDLERDHRPHVSMPQNAN	C-term	CT	
13.51	639.0115	3	1914.0112	0.76	1.17E+07	89	0.0000033	EVEEKRKRKK	NAPPEPVPPRAAPAPTHV	RSPQPPPPAP	VGF	
13.59	609.7757	4	2435.0706	1.26	1.29E+07	65	0.00031	GVGAPGKKR	DMSSDLERDHRPHVSMPQNAN	C-term	CT	
13.68	751.0048	3	2249.9906	0.87	4.65E+06	45	0.03	GVGAPGKKR	DMSSDLERDHRPHVSMPQN	AN	CT	
13.79	659.5054	6	3950.9875	0.31	1.96E+07	119	5.40E-09	Signal	APPGRPEAQPPPLSSEHKEPVAGDAVPGPKDGSAPEVRGA	RNSEPQDEGE	VGF	
14.01	774.6837	3	2321.0277	0.67	7.48E+06	65	0.00033	GVGAPGKKR	DMSSDLERDHRPHVSMPQNA	N	CT	
14.42	734.3739	5	3666.8278	1.45	9.82E+06	72	0.00024	Signal	APPGRPEAQPPPLSSEHKEPVAGDAVPGPKDGSAPEV	RGARN SEPQD	VGF	
14.94	827.7560	6	4960.4862	1.23	1.58E+07	100	5.10E-07	M	SDKPDMAEIEKFDKSKLKKTETQEKNPLPSKETIEQEKQAGES	C-term	TMSB4X	
14.94	633.3134	2	1264.6122	0.03	2.66E+06	69	0.00022	HKQQLERDPR	VKMALQQEGFD	RKKRGYRDIN	PC2	
15.46	823.2621	6	4933.5229	1.22	5.12E+06	131	3.80E-10	M	ADKPDMGEIASFDKAKLKKTETQEKNTLPTKETIEQEKRSEIS	C-term	TMSB10	
15.70	640.3438	2	1278.6721	0.74	7.21E+06	28	0.86	LSRSGGVVKN	NFVPTNVGSKAF-NH2	GRRRDLQA	CGRP	manua
16.14	787.4012	5	3931.9663	0.84	2.37E+06	59	0.0055	Signal	FPKPGGSQDKSLHNRELSAERPLNEQIAEAEEDKI	KKTYPPENKP	SgIII	
16.30	682.3453	5	3406.6865	1.06	4.07E+06	118	5.70E-09	RDFSPSSAKR	QQETAAAETETRTHTLTRVNLESPGPERVW	RASWGEFQAR	VGF	
17.37	848.4229	4	3389.6600	0.73	4.51E+06	93	1.70E-06	RDFSPSSAKR	<qqetaaaetetrthtltrvnlespgpervw< td=""><td>RASWGEFQAR</td><td>VGF</td><td></td></qqetaaaetetrthtltrvnlespgpervw<>	RASWGEFQAR	VGF	
17.60	762.0490	3	2283.1230	0.95	2.56E+06	95	8.50E-07	GSQDKSLHNR	ELSAERPLNEQIAEAEEDKI	KKTYPPENKP	SgIII	
18.64	800.9424	2	1599.8695	0.46	3.70E+06	50	0.011	Signal	VPLPAGGGTVLTKMYP	RGNHWAVGHL	GRP	
19.65	632.5773	4	[2642.3381]	[-1.20]	2.39E+06	[98]	[2.50E-07]	EGSRIAQKR	ACDTATCVTHRLAGLLSRSGGVVKN	NFVPTNVGSK	CGRP	
19.79	819.3663	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	LAALVQDYVQ	CT/CGRP	
20.25	577.2836	3	[1844.8873]	[-1.10]	3.57E+06	[98]	[1.20E-07]	EGSRIAQKR	ACDTATCVTHRLAGLLS	RSGGVVKNNF	CGRP	
20.33	711.9964	8	5687.9079	0.89	2.13E+06	44	0.19	Signal	ERPVFTNHFLVELHKGGEDKARQVAAEHGFGVRKLPFAEGLYHFYHNGLA	KAKRRRSLHH	PC2	manual
20.37	1088.2665	4	4349.0419	-1.15	3.73E+06	112	1.50E-08	EIINSNQVKR	VPGQGSSEDDLQEEEQIEQAIKEHLNQGSSQETDKLAPVS	KRFPVGPPKN	Sgl	
20.60	1144.8639	3	[3547.6323]	[-1.68]	2.29E+06	[62]	[0.00049]	SSLDSPRSKR	CGNLSTCMLGTYTQDFNKFHTFPQTAIGVGAP-NH2	GKKRDMSSDL	CT	
20.84	758.3990	5	[3902.9996]	[-1.10]	5.05E+07	[117]	[2.80E-09]	EGSRIAQKR	ACDTATCVTHRLAGLLSRSGGVVKNNFVPTNVGSKAF-NH2	GRRRDLQA	CGRP	
21.16	830.0130	5	4145.0262	0.58	2.61E+06	95	1.40E-06	EWLKKHDKK	GNKEDYDLSKMRDFINKQADAYVEKGILDKEEAEAI	KRIYSSL	SgIII	
21.50	714.3080	12	8559.6167	-0.94	3.09E+07	8	2.20E+02		intact ubiquitin	C-term	Ubq	standard
21.52	742.1743	5	3705.8346	0.13	2.51E+07	112	2.40E-08	YPGREAQARR	AQEEAEAEERRLQEQEELENYIEHVLLRRP	C-term	VGF	
21.55	893.4781	3	2677.4147	-0.82	4.27E+06	69	0.00031	GVAAPFPKAR	RPESALLGGSEAGERLLQQGLAQVEA-NH2	GRRQAEATRQ	VGF	
21.90	1139.5341	3	[3531.6374]	[-1.57]	2.68E+07	[78]	[1.30E-05]	SSLDSPRSKR	CGNLSTCMLGTYTQDFNKFHTFPQTAIGVGAP-NH2	GKKRDMSSDL	CT	
21.93	1158.8690	3	[3589.6429]	[-1.88]	9.68E+06	[64]	[0.0003]	SSLDSPRSKR	CGNLSTCMLGTYTQDFNKFHTFPQTAIGVGAPG	KKRDMSSDLE	СТ	
22.25	833.0936	6	4992.5178	0.02	7.83E+06	177	1.10E-14	EWLKKHDKK	GNKEDYDLSKMRDFINKQADAYVEKGILDKEEAEAIKRIYSSL	C-term	SgIII	
23.66	1037.1808	6	6217.0396	0.24	3.95E+07	143	2.50E-11	Signal	APFRSALESSPADPATLSED EARLLLAALVQDYVQMKASELEQEQEREGSSLDSPRS	KRCGNLSTCM	CT	
23.69	1010.3493	6	6056.0436	1.41	3.91E+07	156	1.60E-12	Signal	APFRSALESSPADPAT LSED EARLLLAAL VQDYVQMKASELEQEQEREGSR AQ	KRACDTATCV	CGRP	





peptides. Monobasic sites were found in 98 peptides. Overall a total of 373 peptides were shown to have informative cleavage sites to predict the precursor processing in the RSP. As an example, the sequences of CT-derived peptides listed in Tables

I and II were mapped to the precursor sequence (Fig. 5). The map consists of the known major processing products and intermediate products sharing N or C termini, which correspond to the established PC1/3 or PC2 cleavage sites. Consistent with the reported length of its signal peptide (17), no peptide was identified in the first 25 amino acids. Overall this map is reminiscent of CT precursor processing previously elucidated in parafollicular cells of the thyroid gland (17).

DISCUSSION

The most outstanding finding in the present study is that an exocytotic stimulus applied to cultured endocrine cells is highly effective in identifying secretory peptides. Peptide profiles identified with this protocol strongly suggest that most peptides were released from secretory granules on exocytosis (Fig. 4). It should be noted that in a total of 400 identified peptides nearly 97% arose from previously known RSP precursor proteins. This non-invasive approach dispenses with time-consuming procedures such as subcellular fractionation and can be extended to different cell culture models. To the best of our knowledge, this is the first study ever to conduct a comprehensive analysis focused on peptides from the RSP proteins.



FIG. 5. **CT precursor processing deduced by a panel of identified peptides.** The established signal peptide cleavage site (*open arrowhead*) and known processing sites (*closed arrowheads*) are shown across the *top* of the CT precursor with basic residues (*thin black boxes*) and the signal peptide (*hatched box*) indicated. Sequences of identified peptides (*bars*) are detailed in Tables I and II. Major peptides, defined in Table II, are indicated by *gray bars. Black boxes* denote C-terminally amidated residues.

Most peptidomics studies have dealt with endocrine organs or brains, which are considered major sources of peptide hormones or neuropeptides (4, 7–11). It is now recognized that endogenous proteases must be inactivated before tissue extraction to prevent massive production of peptide fragments caused by degradation of abundant intracellular proteins, which hampers MS detection of endogenous peptides (7, 8). For tissue peptidome studies, microwave irradiation before or after decapitation has been proposed to prevent protease activation that is thought to occur immediately after sacrifice (7, 8). Despite these efforts, a limited number of secretory peptides were identified (4, 7–11).

Regarding MSMS of naturally occurring peptides, precursor mass acquisition with conventional mass spectrometers (mass accuracy, \sim 50 ppm) and subsequent filtering in the setting of "no enzyme" very often lead to ambiguous peptide identification (22). To cope with this issue, many peptidomics studies have used in-house databases containing a limited number of entries to identify peptides that would otherwise remain elusive (11, 23). On the other hand, we used the public IPI human database and took into account four variable modifications simultaneously for MSMS interpretation. Because of the mass accuracy of Orbitrap (~2 ppm), a false discovery rate using a decoy database was minimized to 0% for peptide matches above identity thresholds in the Mascot MSMS ion search. This MSMS identification scheme may inevitably miss many peptides, which could be considered identified if a specific database with limited entries was used as in previous peptidomics studies (11, 23). The peptides listed in the supplemental table are all beyond identity thresholds; accepting peptides beyond homology thresholds will allow more than 200 additional secretory peptides to enter the table, with only two different peptides from keratin 8 turning up (data not shown). These examples include neuromedin U 25 (3018.52 Da) and pancreastatin (5076.36 Da) with a Mascot expectation value of 0.063 and 0.43, respectively. In the supplemental table, they are not considered identified because an expectation value for accepting MSMS spectra is the Mascot default significant threshold value of 0.05. Nonetheless we used the

stringent setting (described under "Experimental Procedures") to preclude misleading assignments and to demonstrate that this secretopeptidome shows little contamination by non-secretory components.

In the present study, we made every effort to prevent peptide degradation or chemical modifications (deamidation, methionine oxidation, and pyroglutamination) that may occur during sample preparation. As described under "Experimental Procedures," peptides released during 2 min were immediately subjected to solid phase extraction. Peptide extraction was performed at 4 °C and completed within 20 min after harvesting the supernatant. In addition, lyophilized samples were analyzed by LC-MSMS immediately after reconstitution. Even with these attentive procedures, several clusters of N- or C-terminally truncated peptides that share cleavage sites at the other end were sequenced as reported in previous peptidomics studies on tissue peptidomes (4, 7-11). However, most peptides (30 of 35) dominantly detected in LC-MSMS (Table II, Fig. 3, and supplemental Fig. 1) did not have cleavage sites suggestive of exopeptidase digestion aside from the five CT- or CGRP-derived peptides mentioned under "Results." These five peptides appeared to be N- or C-terminally truncated peptides of major processing products. They have not been reported as major processing products in previous biochemical studies to the best of our knowledge. However, the possibility that their C termini or N termini were generated by unknown endopeptidases (cutting within a peptide) cannot be excluded.

It was unexpected that thymosins and ubiquitin represented major peptides in the TT secretopeptidome. Given a previous report of its storage in adrenal secretory granules and exocytosis-induced secretion (24), ubiquitin may be localized in TT secretory granules and secreted upon exocytosis. The secretory nature of thymosin β -4 has also been reported (25); however, thymosins are not regarded as peptides localized in secretory granules. In any case, the successful identification of intact peptide forms indicates that a majority of peptides may not be affected by exopeptidase digestion.

Gel filtration-based separation caused an increase in the number of sequenced peptides among which several peptides suggestive of the PC-mediated cleavage were identified, such as those from the calcium-binding protein calnuc (supplemental table). Calnuc was identified in a soluble fraction of bovine adrenal secretory vesicles (13), and hence our finding suggests that it is a precursor to unknown bioactive peptides.

To identify unique cleavage sites of RSP precursor proteins, we examined N- and C-terminal flanking sequences of the 400 peptides identified. Overall 152 unique cleavage sites that match PC consensus sites were elucidated of which 105 cleavage sites were conserved consecutive dibasic residues. This finding appears to support the contention revealed by previous studies that the most often encountered PC cleavage sites are conserved paired dibasic sites (1, 2, 19). The observation that a majority of cleavage sites were consistent with PC consensus sites ((R/K) X_n (R/K) where n = 0, 2, 4, or 6) should not be overestimated. For instance, the C-terminal cleavage site of the CGRP precursor (RLAGLLS \downarrow RSG) matches this rule but is not known as a processing site. At present its abundance relative to the longer major product of intact CGRP was unavailable, and therefore it remains to be clarified whether the peptide would represent a major processing product secreted by TT cells. We should also consider that shorter peptides tend to be better ionized and readily detected in mass spectrometry schemes.

Conversely cleavages at non-consensus monobasic sites could represent bona fide initial endoproteolytic cleavage sites. In the present study, CgA-derived peptides (1048.51 and 1161.59 Da) sharing the N-terminal cleavage site (FRAR \$\, AYGF) were identified (supplemental table), suggesting that the single arginine (position 356) is a processing site. The C-terminal cleavage site of the 1914.01-Da peptide (VGF residues 463-481) had an arginine at the P10 position. Indeed they have both been shown to be an authentic recognition site for PC2 using PC2 knock-out mice (26). Another example is the N-terminal cleavage site (LSFR↓ARAY) of the CgA 1275.65- and 1388.73-Da peptides known as the processing site for CgA LF-19 peptide (27). Thus atypical monobasic sites should also be considered potential processing sites for a precursor whose processing remains largely unknown. In this context, the single arginine of VGF (position 212) in FQAR \downarrow MPDS may represent a cleavage site as shown by six peptides (supplemental table). It is envisaged that further detailed analysis of the secretopeptidome could identify processing sites with higher confidence. Although the peptide repertoire from a cancer cell line does not necessarily reflect the in vivo processing pattern of RSP precursor proteins, this will not detract from the significance of our study. Indeed the peptides identified with our approach retain cleavage sites created in the RSP to a degree that allows the accurate prediction of processing sites in known peptide hormone precursors (Fig. 5). In summary, we showed that peptidomics has the potential to identify processing sites of precursors processed in the RSP. By dissecting the secretopeptidome we should have a clearer picture of the precursor processing that actually occurs in the RSP that would also facilitate the discovery of bioactive peptides.

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REFERENCES

- Zhou, A., Webb, G., Zhu, X., and Steiner, D. F. (1999) Proteolytic processing in the secretory pathway. J. Biol. Chem. 274, 20745–20748
- Fricker, L. D. (2005) Neuropeptide-processing enzymes: applications for drug discovery. AAPS J. 7, E449–455
- Brakch, N., Rholam, M., Boussetta, H., and Cohen, P. (1993) Role of beta-turn in proteolytic processing of peptide hormone precursors at dibasic sites. *Biochemistry* **32**, 4925–4930
- Clynen, E., Baggerman, G., Veelaert, D., Cerstiaens, A., Van der Horst, D., Harthoorn, L., Derua, R., Waelkens, E., De Loof, A., and Schoofs, L. (2001) Peptidomics of the pars intercerebralis-corpus cardiacum complex of the migratory locust, Locusta migratoria. *Eur. J. Biochem.* 268, 1929–1939
- Schrader, M., and Schulz-Knappe, P. (2001) Peptidomics technologies for human body fluids. *Trends Biotechnol.* 19, S55–60
- Sasaki, K., Sato, K., Akiyama, Y., Yanagihara, K., Oka, M., and Yamaguchi, K. (2002) Peptidomics-based approach reveals the secretion of the 29-residue COOH-terminal fragment of the putative tumor suppressor protein DMBT1 from pancreatic adenocarcinoma cell lines. *Cancer Res.* 62, 4894–4898
- Svensson, M., Sköld, K., Svenningsson, P., and Andren, P. E. (2003) Peptidomics-based discovery of novel neuropeptides. *J. Proteome Res.* 2, 213–219
- Che, F. Y., Lim, J., Pan, H., Biswas, R., and Fricker, L. D. (2005) Quantitative neuropeptidomics of microwave-irradiated mouse brain and pituitary. *Mol. Cell. Proteomics* 4, 1391–1405
- Boonen, K., Baggerman, G., D'Hertog, W., Husson, S. J., Overbergh, L., Mathieu, C., and Schoofs, L. (2007) Neuropeptides of the islets of Langerhans: a peptidomics study. *Gen. Comp. Endocrinol.* **152**, 231–241
- Cape, S. S., Rehm, K. J., Ma, M., Marder, E., and Li, L. (2008) Mass spectral comparison of the neuropeptide complement of the stomatogastric ganglion and brain in the adult and embryonic lobster, Homarus americanus. *J. Neurochem.* **105**, 690–702
- Bora, A., Annangudi, S. P., Millet, L. J., Rubakhin, S. S., Forbes, A. J., Kelleher, N. L., Gillette, M. U., and Sweedler, J. V. (2008) Neuropeptidomics of the supraoptic rat nucleus. *J. Proteome Res.* 7, 4992–5003
- Wegrzyn, J., Lee, J., Neveu, J. M., Lane, W. S., and Hook, V. (2007) Proteomics of neuroendocrine secretory vesicles reveal distinct functional systems for biosynthesis and exocytosis of peptide hormones and neurotransmitters. *J. Proteome Res.* 6, 1652–1665
- Brunner, Y., Couté, Y., Iezzi, M., Foti, M., Fukuda, M., Hochstrasser, D. F., Wollheim, C. B., and Sanchez, J. C. (2007) Proteomics analysis of insulin secretory granules. *Mol. Cell. Proteomics* 6, 1007–1017
- Gkonos, P. J., Born, W., Jones, B. N., Petermann, J. B., Keutmann, H. T., Birnbaum, R. S., Fischer, J. A., and Roos, B. A. (1986) Biosynthesis of calcitonin gene-related peptide and calcitonin by a human medullary thyroid carcinoma cell line. *J. Biol. Chem.* **261**, 14386–14391
- Zabel, M., Seidel, J., Kaczmarek, A., Surdyk-Zasada, J., Grzeszkowiak, J., and Górny, A. (1994) Hybridocytochemical and immuno-ultrastructural study of calcitonin gene expression in cultured medullary carcinoma cells. *Histochemistry* **102**, 323–327
- Yamaguchi, H., Sasaki, K., Satomi, Y., Shimbara, T., Kageyama, H., Mondal, M. S., Toshinai, K., Date, Y., González, L. J., Shioda, S., Takao, T., Nakazato, M., and Minamino, N. (2007) Peptidomic identification and biological characterization of neuroendocrine regulatory peptide-1 and -2. *J. Biol. Chem.* 282, 26354–26360
- Jacobs, J. W., Goodman, R. H., Chin, W. W., Dee, P. C., Habener, J. F., Bell, N. H., and Potts, J. T., Jr. (1981) Calcitonin messenger RNA encodes multiple polypeptides in a single precursor. *Science* 213, 457–459
- Cameron, A., Apletalina, E. V., and Lindberg, I. (2002) *The enzymology of PC1 and PC2*. In *The Enzymes* (Dalby, R. E., and Sigman, D. S., eds) 3rd Ed., pp. 291–328, Academic Press, NY
- Eipper, B. A., Stoffers, D. A., and Mains, R. E. (1992) The biosynthesis of neuropeptides: peptide alpha-amidation. *Annu. Rev. Neurosci.* 15, 57–85

- Shen, L. P., Pictet, R. L., and Rutter, W. J. (1982) Human somatostatin I: sequence of the cDNA. Proc. Natl. Acad. Sci. U. S. A. 79, 4575–4579
- Minamino, N., Kangawa, K., and Matsuo, H. (1984) Neuromedin C: a bombesin-like peptide identified in porcine spinal cord. *Biochem. Biophys. Res. Commun.* **119**, 14–20
- Brosch, M., Swamy, S., Hubbard, T., and Choudhary, J. (2008) Comparison of Mascot and XITandem performance for low and high accuracy mass spectrometry and the development of an adjusted mascot threshold. *Mol. Cell. Proteomics* 7, 962–970
- Fälth, M., Sköld, K., Norrman, M., Svensson, M., Fenyö, D., and Andren, P. E. (2006) SwePep, a database designed for endogenous peptides and mass spectrometry. *Mol. Cell. Proteomics* 5, 998–1005
- 24. Kieffer, A. E., Goumon, Y., Ruh, O., Chasserot-Golaz, S., Nullans, G., Gasnier, C., Aunis, D., and Metz-Boutigue, M. H. (2003) The N- and

C-terminal fragments of ubiquitin are important for the antimicrobial activities. FASEB J. 17, 776-778

- Bock-Marquette, I., Saxena, A., White, M. D., Dimaio, J. M., and Srivastava, D. (2004) Thymosin beta4 activates integrin-linked kinase and promotes cardiac cell migration, survival and cardiac repair. *Nature* 432, 466–472
- Pan, H., Che, F. Y., Peng, B., Steiner, D. F., Pintar, J. E., and Fricker, L. D. (2006) The role of prohormone convertase-2 in hypothalamic neuropeptide processing: a quantitative neuropeptidomic study. *J. Neurochem.* 98, 1763–1777
- Orr, D. F., Chen, T., Johnsen, A. H., Chalk, R., Buchanan, K. D., Sloan, J. M., Rao, P., and Shaw, C. (2002) The spectrum of endogenous human chromogranin A-derived peptides identified using a modified proteomic strategy. *Proteomics* 2, 1586–1600