Identification of a Gastrin Response Element in the Vesicular Monoamine Transporter Type 2 Promoter and Requirement of 20 S Proteasome Subunits for Transcriptional Activity*

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Vesicular monoamine transporter type 2 (VMAT2) is crucial for accumulation of monoamine neurotransmitters into neuronal secretory vesicles and histamine into secretory granules of the enterochromaffin-like cell in the acid-secreting gastric mucosa. Gastric VMAT2 expression is regulated by the antral hormone gastrin acting at the CCK2 receptor. We demonstrate a gastrin response element as a beta subunit of the 20 S proteasome, PSMB1, as a potential binding partner. In supershift assays, antibodies to PSMB1 and other proteasome beta subunits disrupted gastrin sensitive protein binding to the VMAT2 promoter. Moreover, RNA interference of PSMB1 significantly inhibited gastrin-mediated VMAT2 transcription. These data support the idea that VMAT2 is important for neuronal function comes from studies in VMAT2 knock-out mice. Homozygotes have severely disrupted patterns of feeding and locomotion and are poorly viable. Heterozygotes are grossly normal but have reduced central monoamine levels and are hypersensitive to the locomotor effects of cocaine and amphetamine and are more sensitive to the neurotoxin 1-methyl-(4-phenyl-1,2,3,6-tetrahydropyridine) (MPTP) and to 3,4-dihydroxyphenylalanine (L-DOPA) (5–7). VMAT2 is able to sequester from cytoplasm, toxins, and transmitters that cause Parkinson-like syndromes, and gain of function haplotypes have been reported to confer protection against Parkinson's disease (8). Transporter affinities for serotonin and the catecholamines are in the low micromolar range and are broadly similar for both VMATs. However, only VMAT2 has micromolar affinity for histamine (3). The ability of VMAT2 to accumulate histamine in secretory vesicles is consistent with its localization in central histaminergic neurons and in enterochromaffin-like (ECL) cells of the gastric epithelium, its only non-neuronal location (4). Histamine secreted by the ECL cell is the major stimulant of acid secretion from gastric parietal cells (9, 10), and ECL cell function is tightly regulated by the hormone gastrin acting through the CCK2 receptor (9, 11, 12). Raised plasma gastrin concentrations increase expression of ECL cell genes that are key to histamine synthesis and secretion, including VMAT2 (13). Gastrin stimulates transcription of VMAT2 in gastric epithelial cells through protein kinase C and mitogen-activated protein kinase/extracellular signal-regulated kinase signaling pathways via phosphorylation of CREB (14, 15) and binding of uncharacterised nuclear factors to a GC-rich region of the promoter (15).

Here we report the definition of a novel gastrin-response element (GRE) that is required for gastrin-stimulated but not basal transcription, and the identification in yeast one-hybrid assays of a proteasome beta subunit as part of the response element binding complex. Gastrin-stimulated protein binding to the GRE was time- and dose-dependent and was disrupted by antibodies to proteasome beta, but not other, subunits. RNA interference knockdown of the beta subunit inhibited induction of VMAT2 expression by gastrin independently of proteasome activity. A second gastrin-sensitive gene, TFF1, was unaffected by beta subunit knockdown. These data suggest a previously...
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unrecognized proteasome beta subunit-related mechanism for transcriptional activation of VMAT2.

EXPERIMENTAL PROCEDURES

**Materials**—Antibodies to the proteasome α, β, and regulatory subunits were obtained from Abcam (Cambridge, UK) and from Biomol (Exeter, UK). Amidated, unsulfated heptadecapeptide gastrin (G17) was obtained from Bachem (St. Helens, UK). Predesigned siRNAs were from Ambion (Huntingdon, UK). The proteasome inhibitor MG132 and epidermal growth factor (EGF) was from Merck Biosciences (Nottingham, UK).

**Cell Culture**—AGS cells stably transfected with the human CCK2 receptor, AGS-G17, were maintained as described previously (15) in Ham's F-12 medium supplemented with fetal bovine serum (10% v/v) and 1% penicillin/streptomycin. AR42J cells were maintained in Dulbecco's modified Eagle's medium supplemented with fetal bovine serum (10%) and 1% penicillin/streptomycin and l-glutamine.

**Reporters and Transfection**—DNA fragments corresponding to the proximal 86 or 65 base pairs of the VMAT2 promoter, together with 55 bp of exon1, were generated by PCR from rat genomic DNA using BioXact short DNA polymerase (Bioline, London, UK) and cloned directly into pGEMTeasy (Promega, Southampton, UK). Inserts were removed by digestion with HindIII and XhoI and inserted into the same sites in the linearized firefly luciferase reporter vector pXP2 (16). Reporter constructs containing mutations within the proximal 65 bp of the VMAT promoter were generated by PCR using the wild type construct as template. AGS cells stably transfected with the CCK2 receptor (AGS-G17) (15) were seeded into 6-well plates at a density of 2 × 10^5 cells per well. Cells were transfected 24 h after seeding, with 1 µg of VMAT2 promoter-reporter construct together with 0.5 ng of the constitutively active renilla reporter vector phRL-SV40 (Promega) using Transfast (Promega) as described previously (15). 24 h after transfection, cells were stimulated for 6 h with G17 or vehicle in serum-free medium, then extracted for analysis by dual luciferase assay (Promega). Some cells transfected with nuclear extract (10 µg) in the presence or absence of excess (100×) unlabeled competitor oligonucleotides; for supershift assays, binding reactions were incubated with 2 µl of antibody before addition of radiolabeled probe. DNA-protein complexes were electrophoresed through 6% nondenaturing polyacrylamide gels and the dried gels exposed to phosphor storage screens and visualized with an FX plus molecular imager (Bio-Rad, Hemel Hempstead, UK).

**RNA Interference**—Transfection of siRNAs for PSMB1, PSMB1 (30 nM; Ambion, Huntingdon, UK), or scrambled control was performed on AGS-G17 cells in suspension together with promoter-luciferase constructs (1 µg/well) and Renilla luciferase reporter phRL-SV40 (0.5 ng/well), using Amaxa nucleofection apparatus (Amaxa, Cologne, Germany), solution V, and program B023, according to the manufacturer's instructions. Following transfection, cells were seeded into 6-well plates in full medium and 72 h later stimulated for 6 h with G17 (1 nmol), EGF (9 nmol), or vehicle in the absence of serum and analyzed by dual luciferase assay (Promega). Some cells transfected with siRNA were seeded into four chamber culture slides and 72 h later, processed for immunocytochemistry.

**Immunocytochemistry**—AGS-G17 cells were seeded into four-chamber culture slides at a density of 2 × 10^4 cells per chamber. After 24 h, or 72 h after siRNA transfection, cells were fixed in 4% paraformaldehyde, permeabilized with Triton X-100, and processed for indirect fluorescent immunocytochemistry as described previously (18). PSMB1 was detected using a primary mouse monoclonal antibody (Abcam), PSMB6 by a mouse monoclonal (Biomol), and PSMC1 by a rabbit polyclonal (Biomol). Primary antibodies were used at a dilution of 1:500 and visualized using fluorescein-conjugated
RESULTS

Identification of a Gastrin-sensitive Binding Protein—Previous studies indicated that overlapping AP2 and SP1 sites at position −61 to −51 of the rat VMAT2 promoter were essential for effective gastrin-mediated gene transcription, but binding of an as yet uncharacterized nuclear protein complex to the AP2/SP1 site did not appear to be increased by gastrin (15). In the present study, we extended the analysis to include the region immediately downstream of the AP2/SP1 site and identified a protein complex, whose binding to the promoter was potently and dose-dependently stimulated by gastrin (Fig. 1).

Sequence Requirement for Gastrin-sensitive Protein Binding—To precisely define the sequence required for binding to the VMAT2 promoter of the gastrin-sensitive protein, competition EMSAs were performed. Competitors were synthesized incorporating blocks of three or four transverse base mutations, spanning the entire sequence of the wild type probe, together with a mutant (MT) that disrupted both the AP2 and SP1 binding sites (Fig. 2). Inclusion of excess unlabeled wild type competitor in EMSA reactions disrupted binding of gastrin-sensitive protein to wild type probe. Inclusion of excess unlabeled mutated competitors also disrupted binding, except for mutations in the SP1 site or the immediately adjacent downstream region, suggesting that the sequence −56ccgc-cctccc−47 is likely to be important for binding the gastrin-sensitive protein (Fig. 2).

Relationship with Previously Identified Proteins—The gastrin response element defined in the present study has similarities with GC-rich gastrin-responsive regions identified in other promoters, notably those of the TFF1 and Reg-1 genes (17, 19). To determine whether these promoters could bind the gastrin-sensitive protein identified here, we performed competition EMSAs using sequences corresponding to gastrin-responsive elements of the TFF1 and Reg-1 promoters as competitors. Neither sequence was able to prevent binding of the gastrin-responsive protein to the VMAT2 wild type sequence, suggesting that this protein is different from those mediating gastrin responsiveness of TFF1 and Reg-1 (Fig. 3). Moreover, in supershift assays, binding of the gastrin-sensitive band to the wild type probe was neither supershifted nor disrupted when reactions were incubated in the presence of antibodies to a range of transcription factors including SP1–4, AP2, and MAZ (data not shown).

Protein Binding Is Required for Gastrin-mediated but Not Basal VMAT2 Transcription—Previous studies have demonstrated the importance of SP1 sites in the proximal VMAT2 promoter for basal gene expression in gastric epithelial and neuroendocrine cell lines (14, 15). In the present study we used donkey anti-mouse or anti-rabbit secondary antibodies (Strat-ech, Soham, UK) at 1:200.

FIGURE 1. Identification of a gastrin-sensitive nuclear binding protein. A, gastrin-sensitive sequence of the rat VMAT2 promoter indicating canonical SP1 and AP2 binding sites. B, nuclear extracts (10 μg) from AGS-GR cells exposed to G17 (0.01–5 nM) for 1 h were incubated with radiolabeled probe (10 fmol) corresponding to the sequence shown in A and electrophoresed through 6% non-denaturing gels. The gastrin-sensitive band is indicated by the arrow. C, nuclear extracts (10 μg) from AGS-GR cells exposed to G17 (1 nM) for 0–4 h were processed as described for B.

FIGURE 2. Sequence requirement of gastrin-sensitive binding protein. A, sequence of EMSA probe (SP1/AP2 site in bold) and mutant sequences used in competition assays (mutations are boxed). B, competition EMSA. Nuclear extracts (10 μg) from AGS-GR cells exposed to G17 (1 nM for 1 h) were incubated with radiolabeled wt probe (10 fmol) in reactions that included a 100× excess of unlabeled competitor, as indicated, and electrophoresed through 6% non-denaturing gels.
luciferase reporter analysis to confirm that the SP1 site at position −61 to −56, which forms part of the site for gastrin sensitive protein binding, is required for significant basal VMAT2 expression in AGS-GR cells (Fig. 4). However, disruption of the region immediately downstream of this site, which is also essential for binding of the gastrin-sensitive protein, did not significantly reduce basal expression of VMAT2 (Fig. 4). The data therefore suggest that basal expression is sustainable by binding of nuclear proteins other than the gastrin sensitive band. This is consistent with the identification by EMSA of a number of complexes that bind to the −65 to −37 region of the promoter but that are insensitive to gastrin (Fig. 1).

Expression of the −86 bp VMAT2 promoter was increased around 5-fold in AGS-G_R cells that were exposed to 1 nM G17 for 6 h (control, 100 ± 5.6%; G17, 480 ± 92%, n = 4), and the response was not diminished by truncation of the promoter to −65 bp (Fig. 4). Disruption of either the SP1 site or the adjacent downstream bases (but not the adjacent upstream bases) significantly reduced the response to gastrin. The data are therefore consistent with the notion that gastrin increases VMAT2 expression by inducing binding of nuclear proteins(s) to the sequence −56ccgccccctc−47 in the proximal promoter region.

Identification of the Gastrin-sensitive Protein—Since the gastrin response element identified does not correspond to a recognized transcription factor binding site, and because antibodies to a range of well characterized transcription factors were ineffective in supershift assays, we employed a yeast one hybrid system to identify protein-DNA interactions. The gastrin-sensitive protein originally identified in EMSA using nuclear extracts from AGS-GR cells is also present in nuclear extracts from all cell lines examined in the present study, including the rat pancreatic cell line AR42J, which endogenously expresses the CCK2R. In AR42J cell nuclear extracts, the pattern of binding inhibition with mutant competitors was identical to that seen with AGS-GR cells (data not shown). Because nuclear extracts from AR42J cells demonstrated less additional (non gastrin-sensitive) binding to the VMAT2 promoter, these cells were selected as the source of cDNA for the one-hybrid system.

The DNA bait in the yeast one hybrid analysis consisted of bases −65 to −37 of the rat VMAT2 promoter and included the putative gastrin response element identified above. RNA
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extracted from gastrin-stimulated AR42J cells was used to generated cDNA, which was further purified on Chromaspin TE40 columns; agarose gel analysis indicated a predominance of higher molecular weight products (1–10 kbp).

Following transformation of the yeast strain Y187 with target reporter vector, AR42J cDNA and pGADT7-Rec2, 10 yeast colonies were identified that continued to grow in the presence of high levels (60 mM) 3-AT and that contained an integrated library cDNA insert. The pGADT7-Rec2 vector was rescued from these colonies, transformed into Escherichia coli and multiple clones from each original yeast colony subjected to sequence analysis.

Two independent yeast colonies contained an insert corresponding to the entire coding sequence of the rat proteasome β subunit-type PMSB1. Further positive clones contained sequences corresponding to the TATA box-binding protein-associated factor 12 (TAF12), S-phase kinase-associated protein 1A (p19A), and ubiquitin C (UBC). We selected PSMB1 for further study because of its identification in two independent yeast one-hybrid interactions and because a number of recent studies suggest that it may have a role in transcriptional regulation separate from that of transcription factor proteolysis (20–23).

Proteasome β Subunit Binding to the VMAT2 Promoter—When nuclear extracts from AGS-GR or AR42J cells were incubated in EMSAs with a probe corresponding to bases −61 to −37 of the VMAT2 promoter, binding of the gastrin-sensitive protein complex was disrupted by inclusion of an antibody to PSMB1, and to a second β subunit, PSMB4 (Fig. 5). However, antibodies to an α subunit, PSMA5, and to a subunit of the 19 S regulatory complex, PSMC1, did not affect EMSA binding. Moreover, antibodies to proteins encoded by other positive clones identified in yeast one-hybrid screening (e.g. TAF12) did not disrupt binding either (Fig. 5). Indirect fluorescent immunochemistry confirmed that PSMB1 and PSMB4 (a further proteasome β subunit, PSMB6) were strongly expressed in the nucleus of AGS-GR cells and were also present within the cytoplasmic compartment (Fig. 6). Taken together, these data are consistent with the idea that β subunits of the proteasome form part of the complex that binds to the gastrin response element of the VMAT2 promoter. They also imply that other classes of subunit may not contain the specific binding domains required to form part of the complex.

PSMB1 Is Required for Gastrin-stimulated VMAT2 Transcription—To determine the dependence of gastrin-stimulated VMAT2 expression on individual proteasome subunits, we used RNA interference to knock down expression of PSMB1 in AGS-GR cells. 72 h following nucleofection of AGS-GR cells with PSMB1 siRNA, expression of PSMB1 detected by immunofluorescence was markedly reduced.

FIGURE 6. PSMB subunits are strongly expressed in AGS-GR cell nuclei. AGS-GR cells were fixed and analyzed by fluorescent immunocytochemistry. a–c, ×40; d, anti-PSMB4; e, DAPI; f, overlay. d and f, ×63 deconvolved images; d, anti-PSMB4; e, anti-PSMB1; f, anti-PSMB6.
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(Fig. 7). When AGS-G$_{R}$ cells with PSMB1 knocked down were simultaneously transfected with the −65 bp VMAT2 promoter-reporter construct, luciferase expression in response to gastrin stimulation was significantly reduced, compared with cells treated with scrambled control siRNA. Similar data were obtained with a second PSMB1 siRNA (Fig. 7). In marked contrast, siRNA knockdowns of a regulatory particle (PSMC1) of the 19 S proteasome did not reduce transcription, even though PSMC1 expression was visibly reduced (Fig. 7).

Activity of the Intact Proteasome Is Not Essential for Gastrin-stimulated VMAT2 Transcription—To establish if the effect of PSMB1 is proteasome-dependent, we studied gastrin-stimulated transcription of VMAT2 in the presence or absence of the proteasome inhibitor MG-132. When AGS-G$_{R}$ cells were incubated in the presence of MG-132 (2 µM), the response to gastrin was reduced by 30% compared with that seen in cells incubated with vehicle. There was no further reduction in the presence of 10 µM MG-132 suggesting that the major effect of PSMB1 is independent of proteasome activity (Fig. 8).

PSMB1 Does Not Mediate Transcription of All Gastrin-sensitive Genes—Although known gastrin response elements in the proximal promoters of other genes such as Reg-1 and TFF1 did not bind PSMB1 in EMSAs (Fig. 3), we nevertheless determined whether knockdown of PSMB1 affected transcription of TFF1 stimulated by gastrin or independently by EGF. In contrast to the substantial reduction of VMAT2 transcription in the presence of PSMB1 siRNA, gastrin stimulation of a construct containing 1.4 kb of the human TFF1 promoter was completely unaffected (scrambled control, 13.1 ± 1.9-fold stimulation; PSMB1 siRNA, 14.1 ± 2.8-fold, Fig. 9). Moreover, stimulation of TFF1 transcription by EGF was not significantly reduced by PSMB1 knockdown (Fig. 9).

DISCUSSION

Physiological regulation of gastric acid secretion by the hormone gastrin is critically dependent upon the synthesis, storage, and release of histamine from the ECL cell (9, 10, 13, 24). Expression of genes involved in these aspects of ECL cell function are tightly regulated by gastrin, including the gene encoding VMAT2, which is required for the sequestration of histamine into the secretory vesicles (4, 13, 25). Gastrin stimulates transcription of VMAT2 in part by phosphorylation and
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binding of the gastrin-sensitive band is \(-^{56} \text{ccgcccctc}^{47}\), which includes, but is not restricted to, a canonical SP1 site (Fig. 2), and might therefore be designated a GRE. The relevance of nuclear protein binding to the GRE for gastrin-stimulated VMAT2 transcription is demonstrated by the fact that gastrin-responsiveness of the promoter was significantly reduced by mutations within either the 5’ or 3’ regions of the GRE (Fig. 4). The GRE identified here shares moderate sequence similarity with gastrin-responsive elements in the promoters of other gastrin-sensitive genes such as TFF1 and Reg-1 (17, 19), but in EMSAs these were shown not to bind the gastrin-sensitive protein found in the present study. Similarly, previous studies have demonstrated that gastrin responsive elements found in the promoters of other genes involved in ECL cell histamine biosynthesis and secretion (HDC, chromogranin A) do not bind nuclear proteins involved in gastrin-mediated VMAT2 transcription (15, 26, 27).

The gastrin-sensitive binding complex was initially identified in the gastric cancer cell line AGS, permanently transfected with the CCK\(_2\)R, a system that has been widely used to study mechanisms of gastrin-stimulated gene transcription (see for example, Ref. 28). The nuclear binding protein was, however, identified in a range of cell lines including the rat pancreatic cancer cell line AR42J that endogenously expresses the CCK\(_2\)R (Fig. 5).

A yeast one-hybrid strategy was employed to identify nuclear proteins from AR42J cells that bound the fragment \(-65 \text{ to } -37\) bp of the VMAT2 promoter that contains the novel gastrin response element. From 10 positive clones identified in the one hybrid screen, two contained in frame sequences of the entire coding region of the rat 20 S proteasome subunit PSMB1.

The ubiquitin-proteasome pathway plays a central role in modulating the intracellular levels of wide range of regulatory molecules, generally short-lived, including transcription factors (29). Ubiquitin-mediated degradation of numerous transcription factors, including MyoD (30), Sox9 (31), and IRF-1 (32) is an important regulatory mechanism in many physiological systems. However, repressors of transcription such as inducible cyclic AMP early repressor (33) may also undergo degradation, and in some cases, for example that of the progesterone receptor, the proteasome is essential for effective transcription (34). In fact it is becoming clear that regulation of transcription by the ubiquitin-proteasome system may be effected by a wide range of mechanisms, some of which are independent of proteolytic activity (35–37). Aside from the straightforward degradation of transcriptional enhancers and repressors, mechanisms have also been described that facilitate cofactor exchange (38) and co-translational mRNA processing (39). Studies on non-proteolytic transcriptional mechanisms have focused primarily on the 19 S proteasome components and identified roles for the regulatory complex in the elongation phase of transcription (40, 41) and in histone methylation (43). Moreover, it seems that the 19 S regulatory complex can also target transcriptional co-activators to promoters (44). Both 19 S and 20 S proteasome subunits have been demonstrated to interact with chromatin at the sites of promoters (42, 45), but the nature of the interaction is unclear. The finding that the 20 S and 19 S subunits displayed differential patterns of binding in genome-wide ChIP assays in yeast supports the idea that they

binding of CREB to the proximal promoter region (14, 15), but there is also a requirement for binding of hitherto uncharacterized nuclear factors (15).

In the present study we sought to characterize the unknown factor(s) required for the stimulation of VMAT2 transcription by gastrin. Previous studies had established that the capacity for gastrin-responsiveness resides in the proximal 100 bp of the VMAT2 promoter (14, 15), and in the present study we demonstrated the presence of several nuclear protein complexes that bound to the \(-65 \text{ to } -37\) region of the rat promoter. However, only one such complex showed binding that was time- and dose-dependent increased by G17 (Fig. 1). Mutational analysis was used to show that the minimal sequence requirement for

FIGURE 8. Effect of the proteasome inhibitor MG-132. Gastrin-stimulated activity of the \(-65\)-bp wt VMAT2 reporter construct was determined in AGS-G\(_2\) cells stimulated with G17 (1 nm) for 6 h in the presence of absence of MG-132. Vehicle (0.1% Me\(_2\)SO) or MG-132 (2 or 10 \(\mu\)M) was included 30 min prior to, and throughout stimulation with gastrin. Experiments were performed in triplicate on two separate occasions and results (mean \(\pm\) S.E.) are expressed relative to that of vehicle control (100%).

FIGURE 9. Effect of PSMB1 knockdown on TFF1. A, AGS-G\(_2\) cells were nucleofected with PSMB1 siRNA1 or scrambled control siRNA (SC), together with 1.4 kb of the TFF1 promoter and Renilla control vector. 72 h later, cells were stimulated for 6 h with G17 (1 nm) or EGF (9 nm). Results (mean \(\pm\) S.E., \(n = 3\)) are expressed as percent maximal response (SC).

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may have a degree of independent function during transcription (22). Consistent with this idea, we found that gastrin-sensitive nuclear protein binding to the VMAT2 promoter was disrupted by antibodies to proteasome β subunits, but not by antibodies to an α-subunit, PSMA5, or a regulatory subunit of the 19 S proteasome, PSMC1.

The precise mechanism by which the proteasome regulates transcription of VMAT2 remains to be established, but the finding that PSMB1 siRNA abrogates the gastrin-stimulated transcription suggests that involvement of the 20 S subunit is a requirement. In contrast, transcription was unaffected by siRNA knockdown of the 19 S regulatory subunit PSMC1. Moreover, the proteasome inhibitor MG-132 only moderately reduced VMAT2 transcription, suggesting that the mechanism is chiefly independent of proteasomal function. The possibility arises therefore that the proteasome β subunits might be acting as a docking site for other proteins. In eukaryotic cells, proteasomes are present in both the cytoplasm and nucleus, although the distribution varies with cellular events such as mitosis as well as external stimuli, which may increase nuclear localization (46–50). Proteasomes are reported to be transported unidirectionally from cytoplasm to nucleus (46), and functional nuclear localization signals have been identified on a number of subunits (50, 51). The 20 S proteasome seems to be imported into the nucleus as an immature precursor complex (52), and it has been suggested that in some circumstances, specific individual subunits might be imported preferentially (42). In the present study, strong nuclear staining of three different proteasome β subunits was observed by immunocytochemistry in AGS-GR cells, together with punctuate staining throughout the cytoplasm.

The present findings are consistent with other emerging data on the involvement of the proteasome in enhancement of transcription but extend them in linking this to activation of a GPCR, the CCK₂R. Aside from the ECL cell, the potential for CCK₂R mediated transcription of the VMAT2 gene also arises in the central nervous system where CCK is one of the most highly abundant neuropeptides and exhibits co-localization with monoaminergic neurotransmitters. CCK is equipotent with gastrin at the CCK₂R, which is the predominant CCK receptor subtype in central nervous system (28). Gastrin-stimulated expression of a second gene, TFF1, was unaffected by PSMB1 knockdown, indicating that the mechanism is not involved in all CCK₂R-mediated gene induction. Since TFF1 expression in response to either gastrin or EGF was largely unaffected by PSMB1 knockdown it seems unlikely that reduction of VMAT2 activity was due simply to broad effects on cell function. The extent to which the proteasome might be involved in other cases of GPCR-mediated stimulation of transcription, and with other target genes, remains to be established. However, it is plausible to suppose that the present findings may represent examples of a hitherto unrecognized mechanism for the enhancement of GPCR-stimulated gene transcription.

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