Transcriptional activation of vesicular monoamine transporter 2 in the pre-B cell line Ea3.123

Fiona WATSON¹, Damian G. DEAVALL, Janet A. MACRO, Rachel KIERNAN and Rod DIMALINE

The Physiological Laboratory, University of Liverpool, Crown Street, Liverpool L69 3BX, U.K.

Uptake and storage of monoamines in secretory granules is accomplished by vesicular monoamine transporters, and it is likely that vesicular monoamine transporter 2 (VMAT2) is important for histamine transport *in vivo*. In the present study we have used the pre-B-cell line Ea3.123 to investigate the mechanisms involved in the transcriptional activation of the VMAT2 gene. In Ea3.123 cells, VMAT2 mRNA abundance was increased following mobilization of intracellular calcium, and this increased mRNA expression was paralleled by changes in L-histidine decarboxylase mRNA, suggesting that VMAT2 may be responsible for sequestration of histamine into secretory vesicles in this cell line. We cloned the 5'-flanking region of the VMAT2 gene and determined its transcriptional start site by primer

extension of rat VMAT2 mRNA. There was no TATA or TATA-like sequence upstream of this region; instead there were GC-rich elements, Ca²⁺/cAMP-response-element- and SP1-binding motifs. Approx. 900 bp upstream of the transcriptional start site was a purine-pyrimidine repeat sequence that may form a Z-DNA structure. A series of 5'-deletional VMAT2-promoter segments cloned upstream of a luciferase reporter were capable of driving transcription and indicated the presence of multiple regulatory elements, while stimulation with ionomycin or PMA resulted in an increased level of the transcriptional activity of the 5'-promoter segments studied.

Key words: amine, gastrin, haematopoiesis, luciferase, neuron.

INTRODUCTION

Histamine plays important roles in various physiological systems and pathological reactions, including gastric acid secretion, inflammation and allergy and central neurotransmission [1–6]. It is synthesized from L-histidine by the action of the enzyme L-histidine decarboxylase (HDC), and increased histamine biosynthesis is associated in a variety of situations with increases in HDC mRNA [7–12]. Histamine and other classical neurotransmitters are synthesized in the cytoplasm and require transport into secretory vesicles for regulated exocytotic release and, in some systems, separate vesicular transporters for biogenic amines, acetylcholine and γ -aminobutyric acid ('GABA') have been identified [13–16]. One of two vesicular monoamine transporters, vesicular monoamine transporter 2 (VMAT2), is capable of transporting histamine albeit at lower affinity than other biogenic amines and this transporter has been shown to be localized to the monoaminergic neurons [17], where it has an essential role in maintaining proper neuronal function. VMAT2 has been cloned from both rat and human brain stem, bovine adrenal medulla and a basophilic leukaemia cell line [14,15,18–20], and there is strong evidence that VMAT2 is likely to mediate histamine transport into secretory vesicles of the rat enterochromaffin-like (ECL) cell [9,21–23]. Monoamine stores accumulated by normal VMAT2 function may play significant roles in locomotor stimulation and/or the behavioural reward produced by amphetamines, and malfunctions of monoamine transport have been implicated in psychiatric disease. In addition, mice with genetically impaired VMAT2 expression demonstrate impairment in monoamine storage and release, and their striatal

dopamine nerve terminals have been shown to be more vulnerable to *in vivo* toxicity by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a drug with parkinsonism-producing effects [24,25]. On entry of calcium into chromaffin cells there is increased synthesis of their vesicular monoamine transporter that has been postulated to be due to increased transcriptional activation [26], and recent work has demonstrated that VMAT2 gene expression may be regulated by ovarian hormones [27]. In the present study we demonstrate the co-ordinated up-regulation of mRNA for both VMAT2 and HDC in a pre-B-cell-line Ea3.123 following stimulation with ionomycin. Actinomycin D prevented this up-regulation, indicating that the increased mRNA abundance was the result of increased transcription. In order to characterize this response further, we cloned approx. 2.0 kb of the rat VMAT2 promoter and made 5'-deletional constructs of the promoter to examine regions important for transcriptional activity. We confirmed that the VMAT2 promoter contains no TATA or TATA-like sequence upstream of the start site and that there are likely to be multiple regulatory elements within the promoter region. The experiments demonstrate the up-regulation of VMAT2 reporter expression after stimulation with ionomycin and PMA and provide a model for VMAT2 transcriptional activation that may be valid for a large number of amine-handling cell types.

MATERIALS AND METHODS

Genomic PCR cloning

The promoter region of the rat VMAT2 gene was cloned using a Genome Walker kit (Clontech, Heidelberg, Germany). PCR

Abbreviations used: VMAT2, vesicular monoamine transporter 2; HDC, L-histidine decarboxylase; ECL, enterochromaffin-like; CRE, Ca²⁺/cAMP response element; AP2, activator protein-2; NF, nuclear factor; IL-3, interleukin-3; FBS, foetal-bovine serum; RBL, rat basophilic leukaemia; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

¹ To whom correspondence should be addressed (e-mail watso@liv.ac.uk).

The nucleotide sequence of the clone pV2404-8 has been deposited in the EMBL/GenBank[®]/DDBJ Nucleotide Sequence Databases under the accession number AF047575.

Table 1 Sequences of synthetic oligonucleotide primers used for genomic PCR cloning of the VMAT2 promoter, construction of deletional mutants and primer extension assays

Oligonucleotide	Sequence	Function
AP1	5'-GTAATACGACTCACTATAGGGC-3'	Genomic PCR (forward)
AP2	5'-ACTATAGGGCAGCGTGGT-3'	Nested genomic PCR (forward)
GSP1	5'-TGCTGCACTCTGGCTCGCCTGTGACT-3'	Genomic PCR (reverse)
GSP2	5'-GTTATAGCGCCACACAGCCTGGGTCTCT-3'	Nested genomic PCR (reverse and primer extension)
F1289	5'-GAGCTCTCTAAAGAGAAG-3'	Deletional mutation (forward)
F943	5'-GAGCTCTACTGTCTATCACA-3'	Deletional mutation (forward)
F609	5'-GAGCTCAGAGCCTGCAGTCAG-3'	Deletional mutation (forward)
F223	5'-GAGCTCCTGCGGCTGTGTTCT-3'	Deletional mutation (forward)
R82	5'-CATATGGTCGACCTGCAGG-3'	Deletional mutation (reverse)

Figure 1 HDC and VMAT2 mRNA abundance in Ea3.123 cells stimulated with ionomycin and PMA

Ea3.123 cells were stimulated for 12 h with different concentrations of ionomycin (**a**) and PMA (**c**) prior to extraction of their total RNA and analysis by Northern blotting. Values for HDC and VMAT2 mRNA abundance (mean $\pm$ S.E.M.) represent the results from a minimum of four experiments. In (**b**), Ea3.123 mRNA abundance was determined in response to stimulation with 10^{-6} M ionomycin. The top panel shows changes in VMAT2 mRNA expression with time in untreated (—) and in stimulated (+) cells. The bottom panel shows HDC mRNA expression in untreated (—) and stimulated (+) cells over a similar period of 24 h. In (**d**) HDC and VMAT2 mRNA abundance was determined at time points following the stimulation of Ea3.123 cells with 10^{-7} M PMA. Northern analyses used 5 μ g of RNA per sample.

was performed on adaptor-ligated genomic DNA fragments using primer AP1 (Clontech) and a gene specific primer (GSP1) 5'-TGCTGCACTCTGGCTCGCCTGTGACT-3', corresponding to the 5' untranslated region of the published sequence of rat VMAT2. A further nested PCR was performed on the product, using primer AP2 (Clontech; not the same as AP2 protein) and a second gene specific primer (GSP2), 5'-GTTATAGCGCCACACAGCCTGGGTCTCT-3', starting six bases upstream of

GSP1 (Table 1). Genomic PCR products were subcloned into pGEM-TEasy (Promega, Southampton, U.K.), and the longest clone, pV2404-8, sequenced completely in both directions by an automated dideoxy method. Putative recognition sequences for RNA polymerase II, and transcriptional start sites were identified using the TSSW program [28]. Putative binding sites for transcription factors were identified using Signal Scan [29] and TESS [30].

Preparation of RNA

Total RNA was extracted from rat basophilic leukaemia (RBL-2) cells with guanidinium isothiocyanate and centrifuged through a CsCl cushion as previously described [31]. Total RNA was extracted from Ea3.123 cells using Trizol reagent (GIBCO-BRL) as described in the manufacturer's instructions.

Primer-extension analysis

The transcriptional start site of the rat VMAT2 gene was determined by primer extension of VMAT2 mRNA from rat RBL-2 cells. Oligonucleotide primer GSP2 was end-labelled with [γ - 32 P]ATP using T_4 polynucleotide kinase, and the labelled probe annealed with 40 μ g of total RNA from RBL2 cells. The hybrid was reverse-transcribed with avian-myeloblastosis-virus reverse transcriptase (Promega), incubated with DNase-free RNase A, and the reaction allowed to proceed on a 6%-polyacrylamide/urea sequencing gel. The extended products were sized by comparison with GSP2-primed manual sequencing reactions, using genomic VMAT2 clone as template, run in the same gel. The dried gel was exposed to a phosphor storage screen and the image revealed with a PhosphorImager (Molecular Dynamics, Sevenoaks, Kent, U.K.).

Northern-blot analysis

Samples of total RNA (5 or 10 μ g) were denatured and electrophoresed through 1%-agarose/formaldehyde gels. RNA was electroblotted on to nylon membranes (Nytran 13; Schleicher und Schüll, Dassel, Germany) and immobilized by UV-cross-linking. Membranes were pre-hybridized for at least 1 h at 65 °C, then hybridized overnight with cRNA probes for HDC or VMAT2 [7,9]. Equal transfer and loading was determined by subsequent hybridization with an oligonucleotide probe against the 18 S ribosomal subunit [32]. After hybridization, membranes were washed under high-stringency salt conditions and exposed to phosphor storage screens. Images were revealed and quantified using a PhosphorImager.

Reporter constructs

Genomic DNA corresponding to 1632 bp of the rat VMAT2 promoter together with 82 bp of exon1 was excised from pV₂404-8 at a *Sac*I site within the promoter and the *Sal*I site of pGEM-TEasy, and subcloned into the *Sac*I and *Xho*I sites of the firefly luciferase-reporter vector pGL3-Basic (Promega) to form pV₂1632b. A series of shorter 5' deletional mutants were generated by PCR from pV₂404-8 using forward primers that contained a *Sac*I site beginning at nucleotides -1289, -943, -609 and -223, together with a common reverse primer that contained a *Sal*I site, ending at nucleotide +82 (Table 1). PCR products were directionally cloned between the *Sac*I and *Xho*I sites of pGL3-Basic.

Cell culture

Ea3.123 cells were grown in RPMI 1640 medium supplemented with 10% foetal-bovine serum (FBS), 2 mM glutamine, penicillin/streptomycin (100 i.u./ml), 50 μ M mercaptoethanol and 10% WEHI-3B conditioned media [33]. WEHI-3B cells were grown in 10% FBS, 2 mM glutamine, penicillin/streptomycin 100 i.u./ml. RBL cells were grown in RPMI 1640 medium containing 10% FBS and penicillin/streptomycin (100 i.u./ml). Cells were stimulated as described in the Figure legends. Actinomycin D, ionomycin and PMA were from Sigma.

Transfections

Cells to be transfected were maintained at 1×10^6 /ml, harvested, then resuspended to a final concentration of 10×10^6 /ml in RPMI 1640 medium. For each independent transfection, 1 ml of cell suspension containing 10 million cells was mixed with 20 μ g of the appropriate firefly-luciferase reporter construct and 2.5 μ g of the *Renilla* luciferase (pRL-TK) control vector (Promega). After a 10 min incubation on ice, the cells were electroporated at 300 V and 1250 μ F and then added to 19 ml of RPMI 1640 medium supplemented with 10% FBS, 10% WEHI-3B conditioned media and incubated at 37 °C in 5% CO₂ and grown for up to 48 h. Transfected cells were then harvested and lysed in 1 ml of Passive Lysis Buffer (Promega) and luciferase activity determined using the Dual Luciferase Reporter System (Promega), which allows the sequential and independent measurement of the firefly luciferase activity followed by *Renilla* luciferase activity. Stimulations of transfected cells were as described in the Figure legends, and for each transfection the firefly-luciferase value obtained was expressed as a fraction of the activity obtained with the *Renilla* luciferase control vector. Thus each transfection had its own internal control to combat experimental variability due to cell viability and transfection efficiency.

Statistical analysis

Results were analysed for statistical significance using either the Student's *t*-test for paired data or the Wilcoxon signed rank test as determined by the distribution of the data.

RESULTS

HDC and VMAT2 mRNA abundance in Ea3.123 cells following stimulation

Ea3.123 cells were incubated for 12 h in the presence of various concentrations of ionomycin (Figure 1a) and the levels of HDC and VMAT2 mRNA abundance determined by Northern analyses. Stimulation with 10^{-7} M ionomycin produced small increases

Figure 2 Effect of actinomycin D on VMAT2 and HDC mRNA abundance

Ea3.123 cells were incubated for 6 h in the absence or presence of 10^{-6} M ionomycin prior to the addition of actinomycin D (5 μ g/ml). RNA was extracted at various time intervals following addition of the actinomycin D and analysed by Northern blotting. The results shown in the left-hand panel are HDC mRNA abundance at 18 h following the addition of the actinomycin D with (1) untreated cells, (2) cells + actinomycin D, (3) ionomycin-treated cells and (4) cells in the presence of ionomycin and actinomycin D. The results shown in the right-hand panel is VMAT2 mRNA abundance at 18 h following the addition of the actinomycin D with (1–4) above. The results shown represent means $\pm$ S.E.M. for three independent experiments, and statistical analyses used the paired Student's *t*-test. Significantly decreased responses are as marked with an asterisk (*).

Figure 4 Determination of the transcriptional initiation site of the rat VMAT2 gene, by primer extension analysis

Primer GSP2 (Table 1) was annealed to RNA from RBL2 cells and the sizes of the avian-myeloblastosis-virus reverse-transcriptase-extended products (right lane) were compared with GSP2-primed genomic DNA sequencing reaction mixtures (lanes GATC) included in the same sequencing gel. The arrow indicates the position of the major cDNA product in relation to the genomic DNA sequence, which is consistent with the transcriptional start site predicted by computer analysis. The sequence around the transcriptional start site is shown on the left.

sequence of rat VMAT2 (Table1). Hybridization of this primer to RBL-2-cell RNA and extension with reverse transcriptase produced a major product estimated as 83 bp in size (Figure 4), which is consistent with the transcriptional start site predicted by computer analysis (TSSW) of the VMAT2 promoter. Minor additional bands were revealed, which is in keeping with the multiple transcriptional start sites that are a common feature of TATA-less genes. The region upstream of the putative cap site does not contain a recognizable TATA-box motif or variant; instead there is a G+C-rich region and, further upstream, multiple purine-pyrimidine repeats that may form a Z-structure (Figure 3). At position -26 is a Ca^{2+} /cAMP-response-element (CRE) sequence, as well as adjacent multiple SP1- and AP2-binding sites. In addition, there are several half-sites for binding of the glucocorticoid receptor.

Transcriptional activity of the VMAT2 promoter

The transcriptional activity of the VMAT2 promoter was characterized by ligating selected PCR-generated segments of the 5'-flanking region of the VMAT2 gene upstream of the gene for firefly luciferase in the reporter vector pGL3-Basic and transfecting the resultant constructs together with a *Renilla* luciferase vector into Ea3.123 cells. Transcriptional activity of the constructs was recorded as the ratio of the firefly luciferase (VMAT2 constructs) relative to the *Renilla* luciferase (internal control). As shown in Figure 5, transfection of all the constructs resulted in significant luciferase activity. Transient transfection with the largest construct which encompassed nucleotides -1289 to $+82$ lead to a 2.53 ± 0.95 (mean $\pm$ S.E.M)-fold increase over levels observed with the promoterless pGL3-Basic vector, while trunc-

Figure 5 Analysis of transcriptional activity of the 5'-flanking region of the VMAT2 gene

In (a), Ea3.123 cells were transfected with VMAT2 promoter/luciferase constructs (pV₂1632, pV₂943, pV₂609 and pV₂223; shown schematically on the left) containing selected segments of the 5'-flanking region of the VMAT2 gene. Data are presented as a percentage of the luciferase activity obtained with the promoterless vector pGL3-Basic and are representative of the mean $\pm$ S.E.M. for four independent experiments done in triplicate. The annotated line diagram (b) shows the promoter construct lengths in relation to the major putative transcription-factor-binding sites. Constructs are indicated above the line relative to the position of the predicted transcriptional start site. Putative transcription-factor-binding sites are indicated by letters as follows: C, CRE; S/A, combined SP1 + AP2 site; G, glucocorticoid half-site; S, SP1; A, AP2; N, NF; U, upstream stimulating factor; SIL, putative silencer element.

Figure 6 Stimulation of rat VMAT2 promoter activity by 10⁻⁶ M ionomycin

Ea3.123 cells were transiently transfected with the pV₂943 and pV₂223 luciferase-reporter constructs or the promoterless vector pGL3-Basic for 10 h prior to harvesting the cells and assaying of the cell lysates for luciferase activity. At 6 h prior to harvesting, 10⁻⁶ M ionomycin was added to the appropriate constructs. Values represent means $\pm$ S.E.M. for five independent experiments, and statistical analysis used the paired Student's *t*-test. Statistically significant results are marked with an asterisk (*).

Figure 7 Stimulation of rat VMAT2 promoter activity by 10⁻⁷ M PMA

Ea3.123 cells were transiently transfected with the pV₂943 and pV₂223 luciferase-reporter constructs or the promoterless vector pGL3-Basic for 22 h prior to harvesting the cells and assaying of the cell lysates for luciferase activity. At 18 h prior to harvesting, 10⁻⁷ M PMA was added to the appropriate constructs. Values represent means $\pm$ S.E.M. for six independent experiments, and statistical analyses used the paired Student's *t*-test. Statistically significant results are marked with an asterisk (*).

ation of the 5'-flanking region to -943 bp resulted in a further increase in luciferase expression (5.56 ± 0.1 -fold over basic, mean $\pm$ S.E.M.), indicating the presence of at least one negative regulatory element within the region from -1632 bp to -943 bp. Truncation of the 5'-flanking region to -609 bp led to a 63.3 % decrease in luciferase activity as compared with the -943 bp construct, suggesting that there is a positive regulatory element within the 334 bp from -943 bp to -609 bp of the promoter. Finally, further truncation of the promoter (-223 bp to +81 bp construct) restored the luciferase activity to that observed with the -943 bp construct. Thus the 5'-flanking region of the VMAT2 gene displays transcriptional activity, and deletional analysis suggests that this region is likely to contain multiple regulatory elements (Figure 5).

Increased VMAT2 transcriptional activity following stimulation

Ea3.123 cells that had been transfected with either promoterless vector, construct pV₂943 or pV₂223 for 4 h were incubated in the

presence and absence of the stimuli ionomycin or PMA as described in the legends to the Figures. Stimulation of the constructs with 10⁻⁶ M ionomycin to raise intracellular calcium levels resulted in a significant increase in the transcriptional activity of both construct pV₂943 (5.4-fold increase, $n = 5$, $P < 0.05$) and pV₂223 (7.15-fold increase, $n = 5$, $P < 0.01$) when compared with their basal expression levels. There was no increase in the activity of the promoterless vector (Figure 6). Stimulation with PMA also significantly increased the transcriptional activity of pV₂943 (1.8-fold increase, $n = 6$, $P < 0.05$) and pV₂223 (1.78-fold increase, $n = 6$, $P < 0.05$) over their basal expression levels in the absence of any recognizable change in the expression of the promoterless vector (Figure 7).

DISCUSSION

Cells that synthesize and secrete monoamines require a mechanism to sequester them in secretory granules, and monoamine neurotransmitters are accumulated into synaptic vesicles by one of the two monoamine transporters, VMAT2. [34]. Histamine synthesis is important for a number of biological functions. It occurs in the cytosol and it is probable that this amine is sequestered into secretory vesicles prior to its secretion. The likely candidate for such sequestration of histamine is VMAT2 [17]. Data from this laboratory and others are consistent with a role both for VMAT2 in the transport of histamine into ECL-cell secretory vesicles and for its up-regulation to accommodate the increased histamine biosynthesis and secretion that accompanies ECL-cell stimulation [9,22,23]. In addition, studies on VMAT2 knockout mice have supported the idea that vesicular monoamine transport is important in maintaining neuronal function [24,25]. Homozygote mice devoid of VMAT2 die within a few days of birth, but heterozygote VMAT2+/- mice have dysfunctional monoamine storage and release parameters. Evidence suggests that changes in neurotransmitter storage may regulate behaviour and that VMAT2 may protect against the neurotoxin MPTP, a drug which has a parkinsonism-producing effect. Bone-marrow cells are found in the haematopoietic microenvironment and are excellent inducers of histamine production following stimulation by interleukin-3 (IL-3) and the Ca²⁺ ionophore A23187. These stimuli exert their action by increasing the expression of mRNA encoding HDC and by increasing the activity of the enzyme [11,12,35]. Intracellular calcium levels are likely to play a part in this IL-3 response and, in addition, it has been reported that IL-3-dependent cell lines synthesize histamine on exposure to calcium ionophore [11]. In the present study we have demonstrated the presence of mRNA for VMAT2 as well as HDC in the IL-3-dependent pre-B-cell line Ea3.123. On stimulation of the cells with ionomycin there was a substantial up-regulation of the mRNA for HDC, as has previously been reported. However, we report that there was also a parallel increase in the level of VMAT2 mRNA abundance in ionomycin-treated Ea3.123 cells which was due to increased transcription of the VMAT2 gene. Co-ordinated up-regulation of VMAT2 and HDC mRNA was also observed following the stimulation of the Ea3.123 cells with PMA. This co-ordinated expression of VMAT2 and HDC is consistent with the idea that both are important for histamine synthesis and secretion. In order to investigate the mechanisms regulating VMAT2 transcription we cloned approx. 1800 bp of the rat VMAT2 promoter together with 82 bp of exon 1 and subcloned it into the luciferase-reporter vector pGL3-Basic. Primer-extension analysis confirmed the transcriptional start site, and further characterization of the pV₂1632bp clone indicated that there was no TATA or TATA-like sequence upstream of this. There were, however,

extensive GC-rich elements and SP1-binding motifs, which are important elements for many cellular and viral 'housekeeping genes' that do not contain 'TATA' box sequences. In addition a purine-pyrimidine repeat sequence was found approx. 900 bases upstream of the transcriptional start. This region has the potential to assume a Z-DNA form [36], and these structures may have a role to play in transcriptional regulation [33,37]. There were also CRE and AP2 sites. A protein that binds the CRE element has been characterized and cloned [38] and is one of a family of proteins that are activated by increased cAMP and/or calcium [39]. It is also possible that cAMP may induce cellular activation through AP2 sites, which have also been reported to be involved in responsiveness to PMA, retinoic acid and forskolin [39]. The 5'-flanking region and putative promoter regions of the human [40] and mouse [34] VMAT2 genes have recently been documented and both share features similar to those described here for the rat VMAT2 promoter. In particular, all these promoters contain a perfectly conserved CRE site as well as multiple SP1 and AP2 sites clustered within 300 bp of the transcriptional start site. The rat and mouse also contain putative nuclear factor (NF)-1-binding sites. We generated a series of 5'-flanking deletion constructs of the VMAT2 gene in the luciferase-reporter vector from the clone pV2404-8. All of the constructs generated were capable of driving transcription of the VMAT2 gene in the Ea3.123 cells. Functional analysis of the 5'-flanking region of the VMAT2 gene indicated the presence of multiple regulatory elements within the VMAT2 promoter, which were involved in basal transcriptional activity. The mouse promoter contains a sequence about 750 bp upstream of the transcriptional start site that shares some sequence identity with a number of silencer elements [34,41]. A similar sequence occurs in the rat promoter. However, our present data do not support a major role for this particular element in Ea3.123 cells. In Ea3.123 cells transfected with pV₂943 or pV₂223, elevation of intracellular calcium levels by stimulation with ionomycin or activation of protein kinase C with PMA increased the transcriptional activity of the constructs over basal levels. The increased transcriptional response seen with the smallest of our constructs, pV₂223, following stimulation with either PMA or ionomycin was comparable with that seen for the larger, pV₂943, construct, suggesting that the 223 bp upstream of the transcriptional start site contains the necessary *cis*-regulatory elements for complete responsiveness to these stimuli. This region does in fact contain CRE, AP2 and SP1 DNA-binding motifs which are of principal interest in this regard. Further experiments involving the mutagenesis of these sites within this region of the VMAT2 promoter construct will be required to determine if the transcriptional activity observed in response to ionomycin and PMA stimulation are mediated by similar or distinct transcription factors and *cis*-regulatory elements. Additional work is clearly required not only to elucidate the mechanisms regulating VMAT2 transcription in the Ea3.123 cell line but to determine how VMAT2 gene transcription is regulated in diverse cell types such as monoaminergic neurons and gastric ECL cells.

This work was supported by The Wellcome Trust.

REFERENCES

- Kahlson, G. and Rosengren, E. (1968) *Physiol. Rev.* **48**, 155–196
- Brandes, L. J., LaBella, F. S., Glavin, G. B., Paraskevas, F., Saxena, S. P., McNicol, A. and Gerrard, J. M. (1990) *Biochem. Pharmacol.* **40**, 1677–1681
- Rangachari, P. K. (1992) *Am. J. Physiol.* **262**, G1–G13
- Brandes, L. J., Bogdanovic, R. P., Cawker, M. D. and LaBella, F. S. (1987) *Cancer Res.* **47**, 4025–4031
- Saxena, S. P., Brandes, L. J., Becker, A. B., Simons, K. J., LaBella, F. S. and Gerrard, J. M. (1989) *Science* **243**, 1596–1599
- Tasaka, K., Nakaya, N. and Nonaka, T. (1990) *Agents Actions* **30**, 240–246
- Dimaline, R., Sandvik, A. K., Evans, D., Forster, E. R. and Dockray, G. J. (1993) *J. Physiol. (London)* **465**, 449–458
- Swarovsky, B., Eissele, R., Gross, M., Kortner, G., Koop, H., Arnold, R. and Simon, B. (1994) *Digestion* **55**, 97–102
- Dimaline, R. and Struthers, J. (1996) *J. Physiol. (London)* **490**, 249–256
- Hocker, M., Zhang, Z., Fenstermacher, D., Tagerun, S., Chulak, M., Joseph, D. and Wang, T. C. (1996) *Am. J. Physiol.* **270**, G619–G633
- Dy, M., Arnould, A., Lemoine, F. M., Machavoine, F., Ziltener, H. and Schneider, E. (1996) *Blood* **87**, 3161–3169
- Schneider, E., Pollard, H., Lepault, F., Guy-Grand, D., Minowski, M. and Dy, M. (1987) *J. Immunol.* **139**, 3710–3717
- Alfonso, A., Grundahl, K., Duerr, J. S., Han, H. and Rand, J. B. (1993) *Science* **261**, 617–619
- Erickson, J. D., Eiden, L. E. and Hoffman, B. J. (1992) *Proc. Natl. Acad. Sci. U.S.A.* **89**, 10993–10997
- Liu, Y., Peter, D., Roghani, A., Schuldiner, S., Prive, G. G., Eisenberg, D., Brecha, N. and Edwards, R. H. (1992) *Cell* **70**, 539–551
- Burger, P. M., Hell, J. W., Mehl, E., Krasel, C., Lottspeich, F. and Jahn, R. (1991) *Neuron* **7**, 287–293
- Merickel, A. and Edwards, R. H. (1995) *Neuropharmacology* **34**, 1543–1547
- Surratt, C. K., Persico, A. M., Yang, X.-D., Edgar, S. R. and Bird, G. S. (1993) *FEBS Lett.* **318**, 325–330
- Krejci, E., Gasnier, B., Botton, D., Isambert, M., Sagne, C., Gagnon, J., Massoulie, J. and Henry, J. (1993) *FEBS Lett.* **335**, 27–32
- Howell, M., Shirvan, A., Stern-Bach, Y., Steiner-Mordoch, S., Strasser, J. E., Dean, G. E. and Schuldiner, S. (1994) *FEBS Lett.* **338**, 16–22
- Hakanson, R. (1970) *Acta Physiol. Scand.* **340S**, 1–134
- Weihe, E., Schafer, M. K. H., Erickson, J. D. and Eiden, L. E. (1994) *J. Mol. Neurosci.* **5**, 149–164
- Peter, D., Liu, Y., Sternini, C., De Giorgio, R., Brecha, N. and Edwards, R. H. (1995) *J. Neurosci.* **15**, 6179–6188
- Gainetdinov, R. R., Fumagalli, F., Wang, Y. M., Jones, S. R., Levey, A. I., Miller, G. W. and Caron, M. G. (1998) *J. Neurochem.* **70**, 1973–1978
- Takahashi, N., Miner, L., Sora, I., Ujike, H., Revay, R. S., Kostic, V., Jackson-Lewis, V., Predborski, S. and Uhl, G. R. (1997) *Proc. Natl. Acad. Sci. U.S.A.* **94**, 9938–9943
- Desnos, C., Laran, M. and Scherman, D. (1992) *J. Neurochem.* **59**, 2105–2112
- Rehavi, M., Goldin, M., Roz, N. and Weizman, A. (1998) *Mol. Brain Res.* **57**, 31–37
- Solov'yev, V. V. and Salamov, A. A. (1997) in *Proceedings of the Fifth International Conference on Intelligent Systems for Molecular Biology* (Rawling, C., Clark, D., Altman, R., Hunter, L., Lengauer, T. and Wodak, S. eds.), pp. 294–302, AAAI Press, Menlo Park
- Prestridge, D. S. (1991) *Comput. Appl. Biosci.* **7**, 203–206
- Schug, J. and Overton, G. C. (1997) Technical Report of the Computational Biology and Informatics Laboratory, School of Medicine, University of Pennsylvania, publication no. CBIL-TR-1997-1001-v0.0
- Dimaline, R., Evans, D., Varro, A. and Dockray, G. J. (1991) *J. Physiol. (London)* **433**, 483–493
- Dimaline, R., Evans, D., Forster, E. R. and Dockray, G. J. (1994) *Neurosci. Lett.* **172**, 39–41
- Palacios, R., Henson, G., Steinmetz, M. and McKearn, J. P. (1984) *Nature (London)* **309**, 126–131
- Takahashi, N. and Uhl, G. (1997) *Mol. Brain Res.* **49**, 7–14
- Dy, M., Machavoine, F., Lebel, B., Ichikawa, A., Gastinel, L. N. and Schneider, E. (1993) *Biochem. Biophys. Res. Commun.* **192**, 167–173
- Nordheim, A. and Rich, A. (1983) *Proc. Natl. Acad. Sci. U.S.A.* **80**, 1821–1825
- Nordheim, A. and Rich, A. (1983) *Nature (London)* **303**, 674–679
- Montminy, M. R. and Bilezikjian, L. M. (1987) *Nature (London)* **328**, 175–178
- Faisst, S. and Meyer, S. (1992) *Nucleic Acids Res.* **20**, 3–26
- Xu, W., Liu, L., Moosheuer, K. and Emson, P. C. (1997) *Mol. Brain Res.* **45**, 41–49
- Ishiguro, H., Kim, T. K., Job, T. H. and Kim, K. (1993) *J. Biol. Chem.* **268**, 17987–17994