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Expression and regulation of the bovine vesicular monoamine transporter gene

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In monoaminergic cells, the neurotransmitter is accumulated into secretory or synaptic vesicles by a tetrabenazine- and reserpine-sensitive transporter, catalyzing an H^+/monoamine antiport. The major vesicular monoamine transporter from bovine chromaffin cells was cloned, using sequences common to adrenal medulla and brain rat vesicular monoamine transporters. Its identity was confirmed by peptide sequences, determined from the purified protein. Surprisingly, the bovine adrenal medulla sequence, $bVMAT_2$, is more related to the transporter from human and rat brain than to that from rat adrenal medulla. PCR amplification showed that $bVMAT_2$ is expressed in both adrenal medulla and brain, in contrast with the situation reported in rats, where distinct genes appear to be expressed in brain (SVAT or MAT, now renamed $rVMAT_2$) and in the adrenal medulla (CGAT, now renamed $rVMAT_1$). In bovine chromaffin cells, long-term depolarization by KCl resulted in an increase in the level of $bVMAT_2$ mRNA, in agreement with the previously observed increase in the transporter binding sites, suggesting that a coupling between stimulation, secretion and synthesis changes the composition of the secretory granule membrane.

Chromaffin granule; Neurotransmitter transporter; Stimulation-secretion coupling; Synaptic vesicle

1. INTRODUCTION

Vesicular transporters accumulate neurotransmitters into synaptic vesicles, prior to their release. The most extensively studied transporter of this type is the vesicular monoamine transporter (VMAT) of chromaffin granules, because of the availability of bovine chromaffin granules. Monoamine uptake through the vesicular transporter is driven by the H^+-electrochemical gradient generated by a V-type ATPase located on the same membrane (for review see [1]). The transporter has been characterized pharmacologically with the specific inhibitors reserpine, tetrabenazine, and various derivatives. Ketanserin, an antagonist of 5-HT_2 receptors, also binds to the same site as tetrabenazine [2]. These ligands have been used to purify the transporter in an active [3,4] or inactive form [5]. In addition, a pharmacological analysis of bovine chromaffin cells in culture suggested that the expression of the vesicular monoamine transporter is up-regulated under depolarizing conditions [6].

In rat, vesicular monoamine transporters have been cloned by expression in mammalian cells by two different laboratories [7,8]. Although the existence of multiple VMAT genes was not anticipated since pharmacological and biochemical studies had not revealed any functional or structural heterogeneity among transporters from different tissues [9], Liu et al. [7] reported that rats possess two related genes. Our gene is expressed in adrenal medulla and in PC12 cells (CGAT), while the other is expressed in brain (SVAT). The transporters are not, however, specific for peripheral vs. central nervous system, since Erickson et al. [8] reported that MAT (identical to SVAT), which was derived from the rat basophilic leukemia cell line RBL 2H3, is expressed in the brain and in the stomach. It has therefore been agreed at a recent international meeting to rename these transporters $VMAT_1$ and $VMAT_2$, respectively ('Molecular aspects of neuronal transporters', Strasbourg, September 26–30, 1993).

In the present communication, we describe the cloning of the monoamine transporter from bovine adrenal medulla, which has been extensively analyzed biochemically and pharmacologically, and we show that the corresponding mRNA is upregulated in depolarised chromaffin cells, in culture.

2. MATERIALS AND METHODS

2.1. *RNA purification, cDNA library construction and library screening*

Total RNA was extracted from bovine chromaffin cells cultured for 2 days in the presence of 60 mM KCl, which overexpress the vesicular monoamine transporter [6], using RNazol (Bioprobe, Montreuil, France) according to [10]. Poly(A)$^+$ fraction was purified with oligo

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dT Dynabeads (Biosys, Compiègne, France). cDNA synthesis and cloning were achieved using λZAP II vector with the ZAP-cDNA synthesis kit from Stratagene (La Jolla, USA). This cDNA library (4×10^5 clones) was screened with the oligonucleotide TR_1 deduced from a region highly conserved between the nucleotidic sequences of $rVMAT_2$ and $rVMAT_1$ (corresponding to amino acids 313–325 in $rVMAT_2$ and 317–329 in $rVMAT_1$, located between the 7th and 8th transmembrane segments); TR_1 was labelled with ^{32}P using T_4 polynucleotide kinase and [γ-^{32}P]ATP (6,000 Ci/mmol) (NEN, Du Pont de Nemours, France). Hybridizations were performed in 7% SDS, 0.25 M Na phosphate, 0.25 M NaCl at 53°C overnight. Filters were washed in 1% SDS, 0.2 M Na phosphate, at 53°C. We obtained about 60 clones, all corresponding to the same incomplete cDNA.

2.2. *Obtention of a complete $VMAT_2$ clone by the RACE 5′ procedure*

We followed the procedure described by Frohman [11] and modified by Delort et al. [12]. Briefly, one μg of total RNA from K^+ depolarized chromaffin cells was submitted to reverse transcription using the TA_1 oligonucleotide as a primer in Taq buffer (50 mM KCl, 2.5 mM $MgCl_2$, 0.1 mg/ml BSA, 20 mM Tris-HCl, pH 8.4). Single-stranded cDNA was purified using glass MAX (Gibco BRL). The purified cDNA (1/5 of the preparation) was used directly for dA-tailing with 10 units of terminal deoxynucleotide transferase in 20 μl of twice diluted Taq buffer, containing 0.2 mM dATP (10 min incubation at 37°C). The tailed cDNA (5 μl) was directly used for the first PCR amplification in 50 μl, containing primers TA_4 and R_0 (10 pmol each), dT adaptor (2 pmol), 2.5 units Taq DNA polymerase (Bioprobe Systems), 200 μM dNTP, 150 μg/ml BSA, 16 mM $(NH_4)_2SO_4$, 2.5 mM $MgCl_2$, 20 mM Tris-HCl, pH 8.55. Complementary strands were annealed for 2 min at 45°C and extended for 40 min at 72°C. Thirty cycles (45 s at 94°C, 1 min at 50°C, 3 min at 72°C) were carried out in a Techne PHC3 thermocycler. A second round of amplification was carried out for 30 cycles, adding oligonucleotides R_i and TA_5 or TA_6 to 1/50 of the products of the first amplification. The products were separated by electrophoresis in agarose gel and purified. The largest molecules were cloned in Bluescript vector and sequenced. A complete clone was constructed in the CDM8 expression vector by PCR fusion of RACE 5′ products.

cDNA clones, derived from incomplete clones or obtained by the RACE 5′ procedure, were sequenced by the dideoxy method with Sequenase (USB). Reconstruction of the full sequence in CDM8 vector was achieved by primer walking. Compilation of sequence data and restriction maps was done with the programme written by Bellon [13]. Alignment and screening of protein sequences were done using the GCG package (University of Wisconsin, USA).

2.3. *RT-PCR and competitive PCR*

Reverse transcription was performed with total RNA (1 μg) in 20 μl of Taq buffer containing 7 mM $MgCl_2$, 200 μM dNTP, 1 pmol of TA_1 and 10 units of AMV reverse transcriptase (Promega). The mixture was incubated for 40 min at 42°C and the enzyme was inactivated by incubation at 70°C for 10 min. The RT product was directly transferred to a tube containing 10 units/ml of Taq polymerase and dNTP in Taq buffer without $MgCl_2$ (80 μl final volume).

For competitive PCR, the competitor was a 540 bp fragment, amplified with oligonucleotides TA_3 and TA_4 from the rat SVAT ($rVMAT_2$) clone, a gift from Dr. R.H. Edwards. The rat (competitor) and bovine (target) cDNA were discriminated by restriction with *Bam*HI, which produced fragments of 320 and 220 nucleotides for $rVMAT_2$ and 120 and 420 nucleotides for $bVMAT_2$. Stock solutions of the computer were prepared by serial dilutions from $0.25 \cdot 10^{-18}$ to $2.5 \cdot 10^{-17}$ mol. The reverse transcriptase product was diluted in 450 μl of Taq buffer, 0.2 mM dNTP (50 μCi/ml of ^{32}P dCTP), 10 pmol of each primer, 10 units of Taq polymerase per ml. An aliquot (45 μl) was transferred to each competition tube containing the diluted competitor (5 μl) and then overlaid with mineral oil. After 45 cycles (1 min at 94°C, 1 min at 53°C, 1 min at 72°C), 10 μl of the products were diluted in 20 μl of *Bam*HI buffer and incubated for 1 h. The mixture was analyzed by electrophoresis in 5% polyacrylamide gels; the gels were fixed, dried and the radioactivity quantified with a Phosphorimager (Molecular Dynamics).

2.4. *Protein purification and sequence determination*

The vesicular monoamine transporter was purified from bovine chromaffin granules by the procedure of Isambert et al. [5] and by a technique to be described (Gasnier, B., Sagné, C. and Henry, J.P., in preparation). Peptides generated by CNBr cleavage were separated by reverse phase HPLC on an Aquapore RP-8 column, 100 × 2.1 mm (Brownlee). The peptides were eluted in trifluoroacetic acid (TFA) 0.1% (v/v) with a gradient of 70% acetonitrile (v/v) containing 0.08% TFA, at a flow rate of 200 μl/min. Amino acid sequence determination was performed on an Applied Biosystems sequencer (model 477A) equipped with an on-line phenylthiohydantoin analyser (model 120A).

2.5. *Oligonucleotides*

We used the following oligonucleotides (all in the 5′ to 3′ orientation):

TR_1: GAGCCCACGCTGCCCATCTGGATGATG(C/G)AGACCATGTG
TA_1: ATCCCATACAAAACGCAACATCG
TA_2: CCCAGTGAAGACAAAGACCTCCT
TA_3: ACAGATGACGAGGAGAGGGG
TA_4: CTCCAAAGTTGGGAGCTATGAGT
TA_5: CGGAATTCGACTTCCCCACAAACTCGTA
TA_6: AGGTGGTCGGTGCTGTT
TA_7: CCAGACCACCCCGAGCC
dT adaptor: AAGGATCCGTCGACATCGATAATACGACTCACTATAGGGA $(T)_{17}$
Ro: GAAGGATCCGTCGACATCG
R_i: TCGACATCGATAATACGACTCAC

3. RESULTS

3.1. *cDNA cloning and sequence analysis of a bVMAT cDNA clone*

We purified the monoamine transporter from bovine chromaffin granules to homogeneity and determined

Table I

Peptidic sequences derived from purified chromaffin granule monoamine transporter analysis

Peptide	Determined sequence	Position in the $bVMAT_2$ sequence[a]
N-terminal	ALSxLALLxxLQ	2
CNBr peptide E_9	LLTVVVPIIPSYLYxIExxK	36
CNBr peptide E_{12}	xAFSxSYAFLLIARSLQGI	179
CNBr peptide E_{23}	xVLVGPPFGxVLYE	234

[a] The numbers indicate the position of the N-terminal residue of the peptide in the $bVMAT_2$ sequence, as shown in Fig. 1. Non identified residues are indicated by x.

peptidic sequences from the N-terminus and from CNBr fragments (Table I). Because these sequences contained numerous uncertainties and did not allow us to prepare satisfactory oligonucleotide probes for cloning the transporter, we used a strategy based on the homology between the two rat vesicular amine transporters, $rVMAT_1$ and $rVMAT_2$ [7]. We reconstructed a complete cDNA clone by screening and RACE 5′.

The nucleotide and deduced protein sequences are given in Fig. 1. The predicted protein clearly corresponds to the monoamine transporter which had been purified from bovine chromaffin granules and charac-

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-93                                                cccaagcttctagagatccctcgacctcgacatccattgtgctctaaagc
-43  tagtggatccaggccagaccaccccgagccgagcccaggagtc ATG GCC CTG AGC GAG CTG GCG CTG CTC
  1                                                 M   A   L   S   E   L   A   L   L

 28  CGC CGG CTT CAG GAG AGC CGG CAC TCG CGG AAG CTC ATC CTG TTC ATC GTG TTC CTC GCG
 10   R   R   L   Q   E   S   R   H   S   R   K   L   I   L   F   I   V   F   L   A

 88  CTG CTG CTG GAC AAC ATG CTG CTC ACG GTT GTG GTC CCC ATC ATC CCG AGT TAC TTG TAC
 30   L   L   L   D   N   M   L   L   T   V   V   V   P   I   I   P   S   Y   L   Y

148  AGC ATT GAG CAT GAG AAA GAT GCT CTA CAA ATC CAG ACC ACC AAG CCC GGG CTC ACA GCC
 50   S   I   E   H   E   K   D   A   L   E   I   Q   T   T   K   P   G   L   T   A

208  TCC GCC CCC GGG AGC TTC CAG AAC ATC TTC TCC TAT TAT GAC AAC TCC ACC ATG GTC ACC
 70   S   A   P   G   S   F   Q   N   I   F   S   Y   Y   D   N   S   T   M   V   T

268  GGG AAC AGC ACC GAC CAC CTT CAG GGG GCG CTG GTG CAC GAG GCC ACC ACG CAG CAC ATG
 90   G   N   S   T   D   H   L   Q   G   A   L   V   H   E   A   T   T   Q   H   M

328  GCC ACT AAC TCG TCC TCG GCC TCT TCC GAC TGT CCC AGT GAA GAC AAA GAC CTC CTG AAT
110   A   T   N   S   S   S   A   S   S   D   C   P   S   E   D   K   D   L   L   N

388  GAG AAT GTG CAG GTC GGG CTG CTG TTT GCC TCG AAA GCC ACT GTC CAG CTC CTC ACC AAC
130   E   N   V   Q   V   G   L   L   F   A   S   K   A   T   V   Q   L   L   T   N

448  CCG TTC ATA GGA CTG TTG ACC AAC AGA ATT GGC TAC CCA ATT CCC ATG TTT ACG GGA TTC
150   P   F   I   G   L   L   T   N   R   I   G   Y   P   I   P   M   F   T   G   F

508  TGC ATC ATG TTT ATC TCA ACA GTT ATG TTC GCC TTC TCC CGC AGC TAC GCC TTC CTG CTG
170   C   I   M   F   I   S   T   V   M   F   A   F   S   R   S   Y   A   F   L   L

568  ATC GCC AGG TCC CTG CAG GGC ATC GGT TCC TCC TGC TCA TCT GTA GCT GGC ATG GGC ATG
190   I   A   R   S   L   Q   G   I   G   S   S   C   S   S   V   A   G   M   G   M

628  CTG GCC AGC GTG TAT ACA GAT GAC GAG GAG AGG GGC AAC GCC ATG GGG ATC GCC CTG GGA
210   L   A   S   V   Y   T   D   D   E   E   R   G   N   A   M   G   I   A   L   G

688  GGC CTG GCC ATG GGG GTC CTA GTG GGC CCC CCC TTT GGG AGT GTG CTG TAC GAG TTT GTG
230   G   L   A   M   G   V   L   V   G   P   P   F   G   S   V   L   Y   E   F   V

748  GGG AAG ACA GCT CCG TTC CTG GTC TTG GCT GCC CTG GTG CTC TTG GAT GGA GCC ATT CAG
250   G   K   T   A   P   F   L   V   L   A   A   L   V   L   L   D   G   A   I   Q

808  CTC TTT GTG CTC CAG CCG TCC CGG GTA CAG CCA GAG AGC CAG AAG GGG ACG CCG CTC ACC
270   L   F   V   L   Q   P   S   R   V   Q   P   E   S   Q   K   G   T   P   L   T

868  ACC CTG CTG AGG GAC CCA TAC ATC CTC ATC GCT GCA GGG TCC ATC TGC TTC GCA AAC ATG
290   T   L   L   R   D   P   Y   I   L   I   A   A   G   S   I   C   F   A   N   M

928  GGG ATT GCC ATG CTG GAG CCA GCC CTG CCC ATC TGG ATG ATG GAG ACC ATG TGT TCC CAC
310   G   I   A   M   L   E   P   A   L   P   I   W   M   M   E   T   M   C   S   H

989  AAG TGG CAG CTG GGC GTG GCT TTC TTG CCA GCT AGC GTC TCT TAT CTC ATT GGA ACC AAT
330   K   W   Q   L   G   V   A   F   L   P   A   S   V   S   Y   L   I   G   T   N

1048 GTT TTT GGG ATC CTC GCA CAC AAA ATG GGG AGA TGG CTT TGT GCT CTT CTA GGA ATG ATA
350   V   F   G   I   L   A   H   K   M   G   R   W   L   C   A   L   L   G   M   I

1108 ATT GTC GGA ATG AGC ATT TTA TGT ATT CCT CTT GCA AAA AAC ATC TAC GGA CTC ATA GCT
370   I   V   G   M   S   I   L   C   I   P   L   A   K   N   I   Y   G   L   I   A

1168 CCC AAC TTT GGA GTT GGT TTT GCA ATT GGC ATG GTG GAT TCA TCA ATG ATG CCC ATC ATG
390   P   N   F   G   V   G   F   A   I   G   M   V   D   S   S   M   M   P   I   M

1228 GGC TAT CTG GTC GAC CTG CGG CAC GTG TCG GTC TAT GGG AGC GTG TAC GCC ATT GCC GAT
410   G   Y   L   V   D   L   R   H   V   S   V   Y   G   S   V   Y   A   I   A   D

1288 GTT GCG TTT TGT ATG GGA TAT GCC ATA GGT CCT TCT GCT GGT GGG GCT ATC GCA AAG GCA
430   V   A   F   C   M   G   Y   A   I   G   P   S   A   G   G   A   I   A   K   A

1348 ATT GGA TTT CCA TGG CTC ATG ACA ATT ATT GGA ATA ATT GAT ATT TCT TTT TTT GCT CCT
450   I   G   F   P   W   L   M   T   I   I   G   I   I   D   I   S   F   F   A   P

1408 CTC TGC TTT TTT CTT TCG AAG TCA CCT GCC AAG GAA GAA AAA ATG GCT ATC CTC ATG GAT
470   L   C   F   F   L   S   K   S   P   A   K   E   E   K   M   A   I   L   M   D

1468 CAC AAC TGC CCC ATT AAA ACA AAA ATG TAC ACG CAG AAC AGC AGC CAG TCG CAT CCG ATA
490   H   N   C   P   I   K   T   K   M   Y   T   Q   N   S   S   Q   S   H   P   I

1528 GGT GAG GAT GAA GAA TCT GAA AGT GAC TGA gacccgcaaaagtcctccaagtacctaattgtataaaag
510   G   E   D   E   E   S   E   S   D   /

1597 tgtttccagtgaaatgactcatccagaactgtcttagtcataccactcatccctggtgaaagtcatacaaccaaaggtt
1676 ccttcttttccaggttaatcgatt
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Fig. 1. Complete coding sequence and deduced primary sequence of $bVMAT_2$. The nucleotide sequence corresponds to the reconstructed clone obtained from partial cDNA clones, extending up to nucleotide 337, and completed by RACE 5′. The coding sequence is complete, since it contains the N-terminal peptide sequence, and was able to generate active transport of catecholamines in transfected cells (manuscript in preparation).

terized biochemically, since it contains the N-terminal 26 amino acids identified by Stern-Bach et al. [14], as well as the internal peptide sequences determined by outselves (Table I).

In Fig. 2, this sequence, $bVMAT_2$, is compared with the published sequences $rVMAT_2$, from rat brain [7,8], $hVMAT_2$, from human brain [15], and $rVMAT_1$, from rat adrenal medulla [7]. The sequence of $bVMAT_2$ indicates a general organization similar to that of the other VMAT genes, with 12 putative transmembrane segments, both N- and C-terminal parts of the protein being in the cytoplasm, and a large luminal loop between the first and the second transmembrane segments. Alignment and comparison of the VMAT amino acid sequences indicate that $bVMAT_2$ is more closely similar to $rVMAT_2$ and $hVMAT_2$ (88% and 89% identiy, respectively), previously identified in rat and human brain, than to $rVMAT_1$ (63% identity), obtained from the rat adrenal medulla.

The difference between the $VMAT_2$ and $VMAT_1$ sequences is particularly striking in the N- and C-terminal parts of the protein and for the large intravesicular loop (P42 to G135), which represents the most divergent domain between the four sequences: the loop of $bVMAT_2$ is 59% identical to that of $rVMAT_2$, 72% to $hVMAT_2$, and 22% to $rVMAT_1$. Moreover, this loop contains an hypervariable region, extending from T86 to S115. The sequence of $bVMAT_2$ contains three putative N-glycosylation sites in the large intravesicular loop, instead of four sites for $hVMAT_2$ and $rVMAT_2$. In the putative

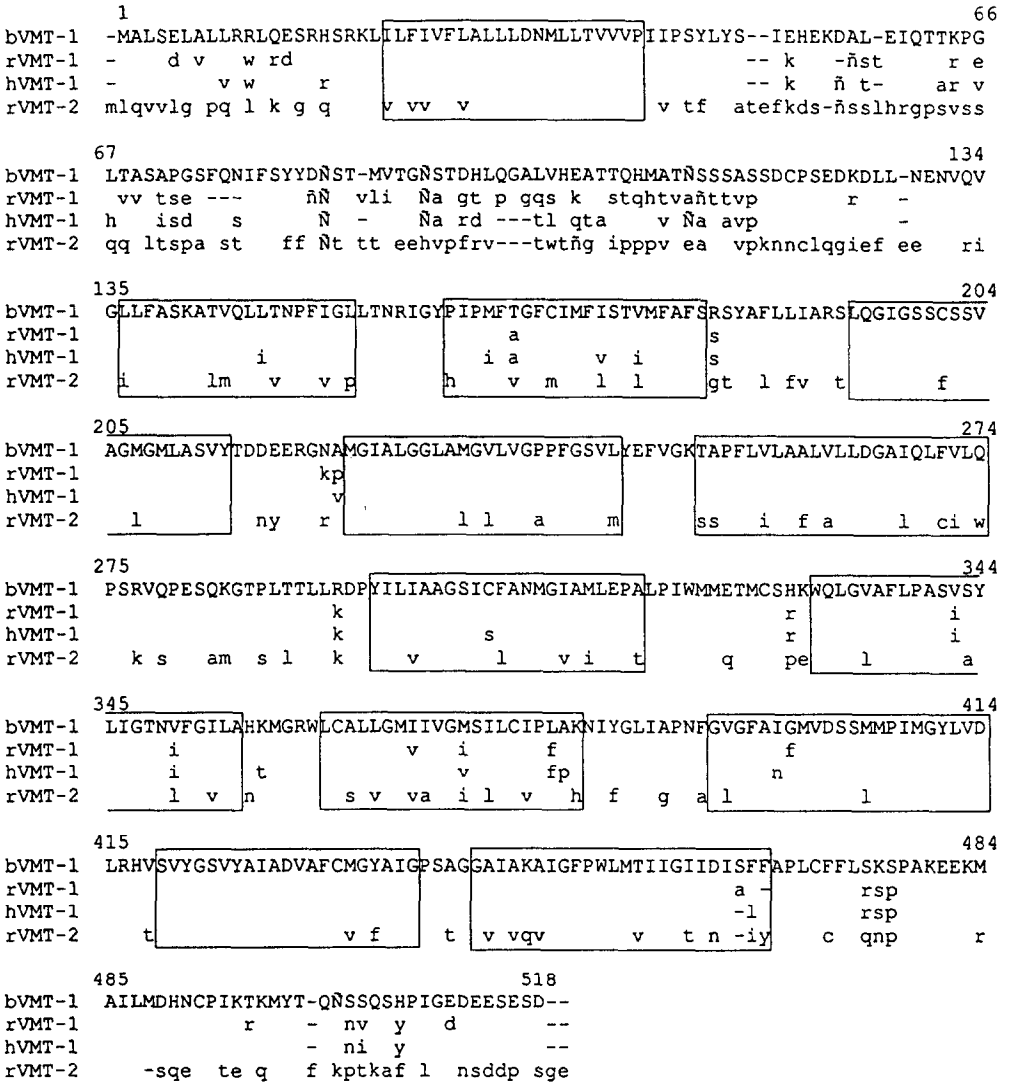


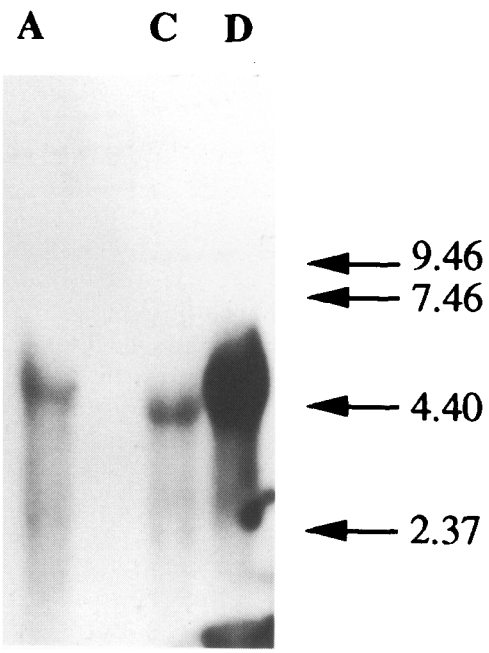
Fig. 2. Comparison of vesicular monoamine transporter sequences. The full sequence of $bVMAT_2$, the vesicular monoamine transporter from bovine chromaffin granules deduced from our cloned cDNA, is shown in capital letters. For the other sequences, only divergent residues are shown, in lower case letters; $rVMAT_2$ and $rVMAT_1$ were deduced from rat cDNA sequences ([7] Genbank accession numbers M97381 and M97380) and $hVMAT_2$ from a human cDNA sequence ([15] Genbank accession number L09118). Putative N-glycosylation sites are indicated in bold type. Putative transmembrane domains are indicated by boxes. Numbers refer to the bovine sequence ($bVMAT_2$). Gaps introduced for alignment are indicated by dashes. Potential N-glycosylation sites are indicated by Ñ for conserved sites and ñ for other sites.

transmembrane segments, the positions of the charged residues are conserved among bovine, human and rat $VMAT_2$ (five acidic residues, D33, D265, D402, D429 and D463, and one basic residue, K141), whereas the $rVMAT_1$ sequence does not contain the last aspartic acid. Finally, three protein kinase C phosphorylation sites are conserved between the three $VMAT_2$ sequences (S18, T156 and S282), whereas only two are present in $rVMAT_1$.

3.2. *Regulation of the expression of $bVMAT_2$*

The presence of mRNA encoding $bVMAT_2$ in the brain, as well as in adrenal medulla, was demonstrated by PCR amplification of reverse transcriptase products, using TA_1 and TA_4 oligonucleotides derived from $bVMAT_2$ (Fig. 3).

In Northern blots, we observed a 4 kb transcript in bovine adrenal gland and in cultured chromaffin cells (Fig. 4). This mRNA was overexpressed in cells cultured for 2 days in the presence of 60 mM KCl, in agreement with the fact that the number of [^{3}H]TBZOH



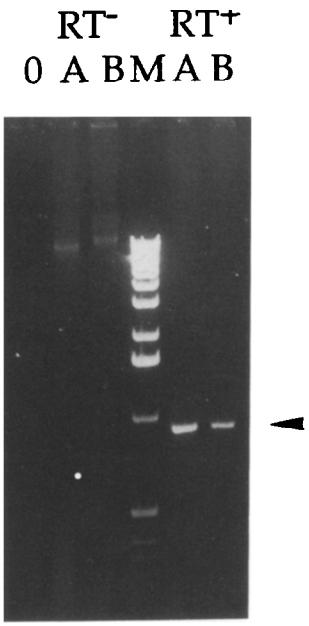


Fig. 3. RT-PCR analysis of $bVMAT_2$ expression in bovine adrenal medulla and brainstem. RNA (4 μg) from adrenal medulla (A) or brainstem (B) were incubated with primer TA_1 in the presence (RT^+) or absence (RT^-) of reverse transcriptase. A control incubation was performed without RNA (○). Aliquots (1/2 of the incubation) were subjected to PCR amplification (35 cycles) with oligonucleotides TA_1 and TA_2 and analyzed by electrophoresis in agarose. M indicates the size markers (one kb ladder); the arrow indicates the amplification product (946 bp).

4. DISCUSSION

We obtained a cDNA clone encoding the vesicular monoamine transporter from bovine chromaffin granules, $bVMAT_2$, since the protein sequence derived from $bVMAT_2$ clone contains the peptides determined by sequencing the bovine protein obtained by ourselves as well as by another group [14]. Surprisingly, $bVMAT_2$ is closely related to $hVMAT_2$ [9] obtained from a brain library, and to $rVMAT_2$ [7,8] also obtained from brain and found not to be expressed in rat adrenal medulla, but it is more distant from $rVMAT_1$, which is derived from PC12 cells. Comparison of the four sequences clearly shows the existence of two transporter types, now named $VMAT_2$ and $VMAT_1$, thus avoiding any reference to any tissue specificity.

Our results clearly show that the two transporters, $VMAT_2$ and $VMAT_1$, are not restricted to either the central or the peripheral nervous systems. In bovine, the $bVMAT_2$ gene is expressed in both brain and adrenal medulla. In addition, we found that another gene, possibly related to $rVMAT_1$, is also expressed in the bovine adrenal medulla (data not shown). Thus, at least two genes encode vesicular monoamine transporters, but their precise relationships remain to be understood.

Analysis of [^{3}H]TBZOH binding showed that the level of the vesicular transporter is increased in chronically depolarized chromaffin cells [6]. We now show

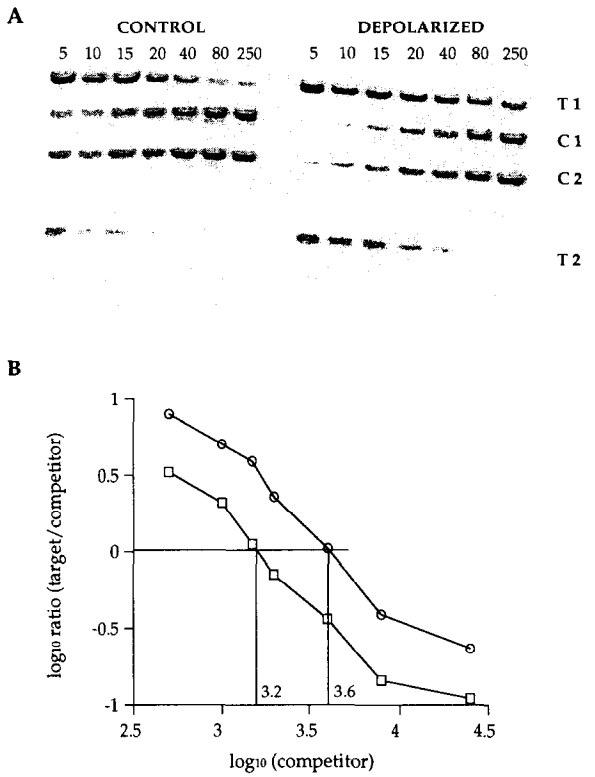


Fig. 5. Competitive reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of $bVMAT_2$ induction in K^+-depolarized chromaffin cells. cDNA originating from bovine chromaffin cells cultured in standard or in K^+-containing medium were amplified together with serial dilutions of a fragment of $rVMAT_2$, acting as a competitor. The single-stranded cDNA obtained by reverse transcription of chromaffin cell RNA was mixed with various concentrations of $rVMAT_2$, and subjected to amplification. The amplification products, obtained with the same primers, were of equal lengths but could be distinguished by the size of fragments obtained after cleavage with *Bam*HI. (A) Scanning of the dried gel: the target, $bVMAT_2$, and the competitor, $rVMAT_2$, produced bands T_1, T_2 and bands C_1, C_2, respectively. The number of competitor copies (× 100) used in the amplification, is indicated for each lane. (B) Logarithmic plot of the data: control cells (□); cells which had been depolarized for two days, in the presence of 60 mM KCl (○).

that the level of mRNA encoding $bVMAT_2$ is also increased under these conditions. The increase in $bVMAT_2$ mRNA, as measured by competitive PCR, is similar to that reported for tyrosine hydroxylase [17] or components of the granule matrix such as proenkephaline [18], which are regulated at the transcriptional level. This is the first time that such a regulatory process is described for a neurotransmitter transporter. If the vesicular uptake is rate-limiting under conditions of prolonged secretion, as discussed in detail elsewhere (Desnos, C., Laran, M.P. and Henry, J.P., manuscript in preparation), increased expression of the vesicular monoamine transporter would serve an adaptive purpose, and could be important from a clinical point of view. It has been proposed that neurodegenerative diseases, such as Parkinson's disease, are accompanied by a prolonged overstimulation of the remaining neurons. In a clinically silent phase, masking the evolution of the degenerative process, these neurons would thus adapt by increasing the synthesis of limiting components, perhaps including the vesicular transporter.

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REFERENCES

[1] Johnson, R.G. (1988) Physiol. Rev. 68, 232–306.
[2] Darchen, F., Scherman, D., Laduron, P. and Henry, J.P. (1988) Mol. Pharmacol. 33, 672–677.
[3] Stern-Bach, Y., Greenberg-Ofrath, N., Flechner, I. and Schuldiner, S. (1990) J. Biol. Chem. 265, 3961–3966.
[4] Vincent, M.S. and Near, J.A. (1991) Mol. Pharmacol. 41, 889–894.
[5] Isambert, M.F., Gasnier, B., Botton, D. and Henry, J.P. (1992) Biochemistry 31, 1980–1986.
[6] Desnos, C., Laran, M.P. and Scherman, D. (1992) J. Neurochem. 59, 2105–2112.
[7] Liu, Y., Peter, D., Roghani, A., Schuldiner, S., Privé, G.G., Eisenberg, D., Brecha, N. and Edwards, R.H. (1992) Cell 70, 539–551.
[8] Erickson, J.D., Eiden, L.E. and Hoffman, B. (1992) Proc. Natl. Acad. Sci. USA 89, 10993–10997.
[9] Henry, J.P. and Scherman, D. (1989) Biochem. Pharmacol. 38, 2395–2404.
[10] Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
[11] Frohman, M.A., Dush, M.K. and Martin, G.R. (1988) Proc. Natl. Acad. Sci. USA 85, 8998–9002.
[12] Delort, J., Dumas, J.B., Darmon, M.C. and Mallet, J. (1989) Nucleic Acids Res. 17, 6439–6448.
[13] Bellon, B. (1988) Nucleic Acids. Res. 16, 1837–1846.
[14] Stern-Bach, Y., Keen, J.N., Bejarano, M., Steiner-Mordoch, S., Wallach, M., Findlay, J.B.C. and Schuldiner, S. (1992) Proc. Natl. Acad. Sci. USA 89, 9730–9733.
[15] Surratt, C.K., Persico, A.M., Yang, X., Edgar, S.R., Bird, G.S., Hawkins, A.L., Griffin, C.A., Li, X., Jabs, E.W. and Uhl, G.R. (1993) FEBS Lett. 318, 325–330.
[16] Gilliand, G., Perrin, S., Blanchard, K. and Bunn, H.F. (1990) Proc. Natl. Acad. Sci. USA 87, 2725–2729.
[17] Stachowiak, M.K., Hong, J.S. and Viveros, O.H. (1990) Brain Res. 510, 277–288.
[18] Eiden, L.E., Giraud, P., Dave, J.R., Hotchkiss, A.J. and Affolter, H.U. (1984) Nature 312, 661–663.