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Novel, Testis-Specific mRNA Transcripts Encoding N-Terminally Truncated Choline Acetyltransferase

PETER LÖNNERBERG AND CARLOS F. IBÁÑEZ*

Division of Molecular Neurobiology, Department of Neuroscience, Karolinska Institute, Stockholm, Sweden

ABSTRACT Previous studies reported the presence of choline acetyltransferase (ChAT) mRNA and protein in the mammalian testis. We have now found that none of the ChAT mRNAs produced in the testis is capable of encoding a full-length ChAT protein. Two ChAT cDNAs were isolated from an adult rat testis cDNA library encoding N-terminally truncated ChAT proteins of 450 and 414 amino acids (aa), respectively, the former containing a novel N-terminal extension of 69 residues. Rapid Amplification of cDNA Ends (RACE) analysis revealed a complex pattern of 5′ untranslated mRNA termini generated from the ChAT gene locus in the testis, all representing truncated versions of the ChAT enzyme. Two of these proteins were produced in transfected fibroblasts and found to lack ChAT activity. Neither did they show binding to the ChAT substrates, acetyl CoA and choline, in a competition assay. These results indicate that mammalian testis lacks a bona fide ChAT enzyme but expresses truncated ChAT proteins with a possible unique function to the testis. *Mol. Reprod. Dev. 53:274–281, 1999.* © 1999 Wiley-Liss, Inc.

Key Words: cholinergic; gene expression; spermatozoa

INTRODUCTION

Several neurotransmitters and neurotransmitter-synthesizing enzymes originally characterized within the nervous system have later been found in other tissues (Persson et al., 1990a). Among these, are the opioid peptide precursors pro-opiomelanocortin, prodynorphin, and preproenkephalin (Tsong et al., 1982; Kilpatrick and Rosenthal, 1986; Douglass et al., 1987). Expression of the genes encoding gastrin, glutamic acid decarboxylase, and cholecystokinin have also been described in the testis (Persson et al., 1989; Persson et al., 1990; Schalling et al., 1990). A testis-specific mRNA transcript of the neuronal nitric-oxide synthase has been reported (Brenman et al., 1997; Wang et al., 1997), and several other genes have been shown to exhibit unique mRNA variants in the testis, for example the genes for lutenizing hormone β-subunit (Zhang et al., 1995), aspartate aminotransferase (Toussaint et al., 1994), cAMP response element binding protein and modulator (Waeber et al., 1991; Walker et al., 1994), and adenosine deaminase (Meng et al., 1997).

Choline acetyltransferase (ChAT) (EC 2.3.1.6) is the enzyme catalyzing formation of the neurotransmitter acetylcholine from acetyl coenzyme A (AcCoA) and choline. ChAT mRNA and protein is found in cholinergic neurons throughout the nervous system. ChAT and acetylcholine have in addition been demonstrated in some non-neuronal mammalian tissues (Rama Sastry and Sadavongvidad, 1979), for instance the epithelial cells of rat and man (Klapproth et al., 1997). Acetylcholine, as well as ChAT enzymatic activity, have been detected in spermatozoa (Bishop et al., 1976; Stewart and Forrester, 1978), and were implicated in sperm motility. It has been shown that ChAT inhibitors and acetylcholine agonists and antagonists reduce or influence sperm motility (Harbison et al., 1976; Rama Sastry et al., 1981; Dwivedi, 1983). It has been argued that ChAT activity in spermatozoa could be accounted for by the high content of carnitine acetyltransferase of these cells, since this enzyme is also able to synthesize acetylcholine (Goodman and Harbison, 1981; Goodman et al., 1984). In later work, ChAT mRNA was detected in the testis by Northern blot and in situ hybridization, and ChAT immunoreactivity was seen in human spermatozoa (Ibáñez et al., 1991). Immunoreactivity was restricted to a double band in the post-acrosomal region and to the annulus of the tail midpiece of human spermatozoa (Ibáñez et al., 1991). In rat testis, ChAT mRNA level was very low until 24 days of age, then increased and peaked at 32 days of age, after which it decreased to adult levels (Ibáñez et al., 1991).

The rat ChAT gene spans at least 64 kb of DNA and consists of three 5′ non-coding exons, followed by 14 coding exons (Hahn et al., 1992; Kengaku et al., 1993). Each of the 5′ non-coding exons has a unique promoter, designated R, N, and M, respectively. Through differential splicing in the 5′ non-coding region at least five different mRNAs are produced, referred to as R1-, R2-, N1-, N2-, and M-type corresponding to the respective promoters used (Kengaku et al., 1993). In addition to ChAT, the gene encoding vesicular acetylcholine transporter (VAChT) is located in the same locus, within the

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*Correspondence to: Carlos F. Ibáñez, Division of Molecular Neurobiology, Department of Neuroscience, Karolinska Institute, S-171 77 Stockholm, Sweden.

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intron between the R- and N-type exons of the ChAT gene (Bejanin et al., 1994; Erickson et al., 1994). The VAChT gene is transcribed both from its own promoter, and from the R-type ChAT promoter through differential splicing (Bejanin et al., 1994). ChAT mRNA in rat brain is approximately 4.0 kilobases (kb), while in rat testis two ChAT mRNA species of 3.5 and 1.3 kb have been described (Ibáñez et al., 1991). Only the longer of these two mRNAs could potentially encode a full length ChAT enzyme, although this has never been investigated.

In this study, we have analyzed the transcriptional unit of the ChAT gene in testis. We describe novel ChAT mRNA transcripts encoding truncated ChAT proteins lacking choline acetyltransferase activity, the only products of the ChAT gene in testis, raising the possibility that these related products have a unique, yet unknown, function in this organ.

MATERIALS AND METHODS

cDNA Synthesis and Library Screening

Testes were dissected from 150–200 g adult Sprague-Dawley rats. Polyadenylated RNA was prepared by CsCl gradient centrifugation followed by selection on an oligo(dT)-cellulose column as described previously (Ibáñez and Persson, 1991). A cDNA library was made in the λZAP vector from 4 μg of polyadenylated RNA and random hexamers as primers for first strand synthesis using the λZAP cDNA synthesis kit (Stratagene). The library was packed into phages with a kit from Promega. The library was plated and screened with a 513 basepair (bp) *PstI* fragment corresponding to bp 1,157 to 1,670 of rat ChAT cDNA (Brice et al., 1989) as probe. Positive clones were sequenced by the dideoxy sequencing method. The expression construct for tagged proteins from clone λ4 was made by PCR amplification from 200 ng of cDNA plasmid with Vent polymerase (New England Biolabs) and the primers 5′-CAGCGGGCAGCGGCCACCATGG-3′ (the first codon underlined) and 5′-GGCTCGAGCTAAGCGTAGTCCGGAACGTCATATGGGTAGTGG CTGGAGTCAAGATTGCTTG-3′. The amplified product, as well as clone λ6 and neuronal ChAT, were cloned into the pcDNA3 vector (Invitrogen) for expression studies.

PCR Amplification of Novel 5′ Ends and Ribonuclease Protection Assays

5′-Rapid Amplification of cDNA Ends (RACE) was performed using the Marathon cDNA amplification kit (Clontech) with 1 μg of polyadenylated adult rat testis RNA and the downstream primers 5′-CTGAGCTCTCCTGTGCCTGGACCATC-3′ and 5′-AAGGATCCAGGCATACCAGGCAGATGCAGCGCTC-3′. PCR was run for 30 cycles of 94°C × 1 min, 62°C × 30 sec, 68°C × 4 min and then for another 15 cycles for the second downstream primer on a 1:50 dilution of the first reaction. The products were cloned into pBSKS+ (Stratagene) and sequenced. Ribonuclease Protection Assays (RPA) were performed with the RPA II kit (Ambion) on 20 μg of total RNA from rat tissues.

Cell Culture and Transfection

COS cells were grown in DMEM with 10% of Fetal Calf Serum supplemented with 2 mM glutamine, 100 U/ml penicillin, and 100 mg/ml streptomycin (Gibco BRL) at 37°C in a 5% CO_2 atmosphere. Cells grown to approximately 70% confluency were transfected according to the calcium phosphate procedure. Forty-eight hours after transfection cells were harvested in a lysis buffer containing 10 mM sodium phosphate buffer pH 7.4, 10 mM EDTA and 0.05% Triton X-100. ChAT activity was measured according to Fonnum (1975) with minor modifications. The standard 39 μl reaction contains 10 mM sodium phosphate buffer pH 7.4, 10 mM EDTA, 0.27 M sodium chloride, 9 mM choline chloride, 20 μM acetyl coenzyme A, and 90 μM eserine. For background determination, the eserine was replaced by 4.5 mU of acetylcholine esterase. In the experiments with limiting substrate concentrations, the different substrates were diluted as indicated.

In Vitro Translation and Western Blot

Recombinant protein was produced with a reticulocyte lysate in vitro translation kit (Promega). The reaction was run on a 10% SDS-polyacrylamide gel, dried and exposed to a phosphorimager screen (Molecular Dynamics). For western blotting, the gels were transferred to a nitrocellulose filter and processed for immunodetection according to standard procedures using an anti-HA monoclonal antibody (Boehringer Mannheim) diluted 1:5,000 in TBST. After overnight incubation the filter was washed four times in TBST, incubated 1 hr with an alkaline phosphatase-conjugated anti-mouse antibody diluted 1:10,000, rinsed, developed by chemifluorescence (Amersham), and scanned in a Storm 840 fluorimager (Molecular Dynamics).

RESULTS

Full-Length Neuronal ChAT Is Not Expressed in the Mammalian Testis

A 370 bp *MluI-ApaI* genomic fragment spanning the 5′ end of the R-type exon was used as probe in Ribonuclease Protection Assays (RPA). This probe detected R-type neuronal mRNAs from two different transcription initiation sites in spinal cord and cortex, and showed that R-type neuronal ChAT mRNA is not expressed in the testis (Fig. 1). RPA analysis using consecutive downstream riboprobes spanning exon sequences starting at the translation initiation site were used to map the overlap between neuronal (nChAT) and testis ChAT mRNAs. Several of the most 5′ probes failed to detect ChAT mRNA in testis (data not shown). The most 5′ sequence that was fully protected in hybridizations to testis mRNA corresponded to a fragment spanning positions 1,157 to 1,670 of the R-type rat nChAT (Brice et al., 1989) (data not shown). These data suggested that full-length ChAT mRNA as found in brain is not expressed in the mammalian testis.

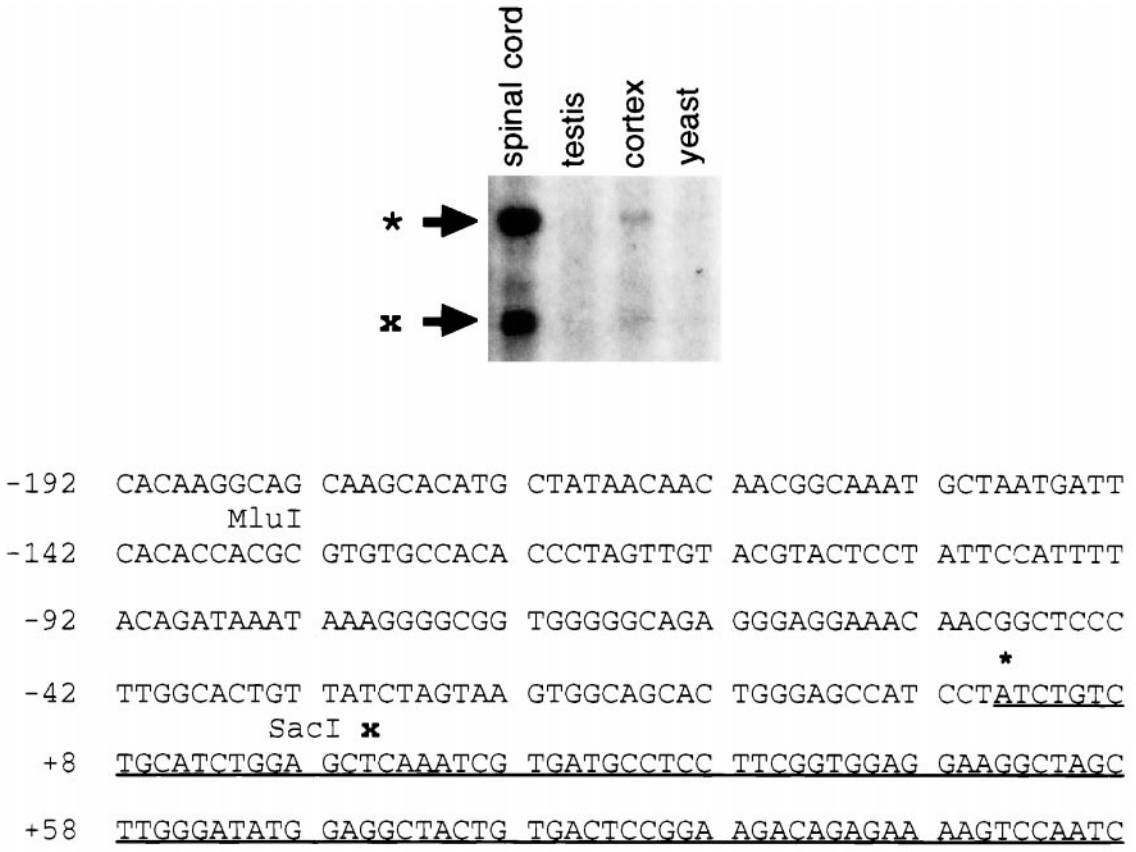

Fig. 1. Ribonuclease protection assays of neuronal ChAT mRNA. 5 µg of polyadenylated mRNA from the indicated tissues was hybridized to a ^{32}P-labeled RNA probe corresponding to a 370 basepair *MluI-ApaI* sequence spanning the transcription start site of R-type neuronal ChAT, and the protected products were separated with acrylamide gel electrophoresis. DNA sequence reactions of plasmids with known sequence were run in parallel to determine the sizes of protected bands (not shown). Below is shown part of the R type cDNA 5′ sequence. The nucleotides corresponding to the protected bands are indicated (* and X). The R type first exon is underlined and selected restriction sites shown.

Cloning of Testis Specific ChAT cDNAs

In order to characterize ChAT mRNAs expressed in testis, we isolated ChAT cDNAs from a random primed cDNA library from adult rat testis using a 513 bp PstI fragment corresponding to positions 1,157 to 1,670 of the R-type rat nChAT cDNA as probe (Brice et al., 1989). Out of 1×10^6 plaques, three positives were found, denoted λ4, λ6, and λ13. Clone λ13 comprised positions 1,186 to 1,912 of the previously reported nChAT sequence. On the other hand, clones λ4 and λ6 had unique 5′ flanks, and a common extended 3′ sequence (Figs. 2 and 3). Clone λ4 was 3,247 bp long and had a unique 998 bp 5′ sequence upstream of exon 8 of the R-type nChAT cDNA (Fig. 3). The longest open reading frame (ORF) of this cDNA beginning with a good Kozak consensus sequence (AAGACATGGGC) (Kozak, 1996) predicted a truncated ChAT protein of 450 amino acids (aa), of which the first 69 are not present in nChAT (Fig. 3). The 3′ non-coding region of this clone was long and extended 1,103 bp past the stop codon. The λ6 clone was 1,854 bp long and had a unique 120 bp 5′ end upstream of exon 7 of the nChAT cDNA (Fig. 3). An acceptor splice-site was found immediately upstream of ChAT exon 7 in clone λ6, suggesting that sequences of intron 6 of nChAT form part of the 5′ flank of this clone. The most 5′ ATG with an ORF is located at position 82 within exon 7 of nChAT. Translation from this start site, which is flanked by a good Kozak consensus sequence (TCAAAATGGCG) would produce a truncated ChAT protein of 414 aa, corresponding to amino acids 261 to 644 of nChAT (Fig. 3).

A Complex 5′ Flank Pattern in Testis ChAT mRNAs

In order to further characterize testis-specific 5′ ChAT mRNA sequences, RACE was performed on testis mRNA (see Materials and Methods). Several new 5′ flanking mRNA sequences were found, some of which corresponded to differentially spliced mRNAs (Fig. 2). Four RACE clones (1B, 1T, 1W, 2B) were identical to phage clone λ6. Two other clones (1J, 1O) had a novel 185 bp 5′ end upstream of the clone λ4 sequence starting at bp 908. At position 907 of clone λ4, a potential acceptor splice-site is located, another indication of differential splicing. The remaining four RACE products isolated (1E, 1F, 1P, 1U) were all colineal with

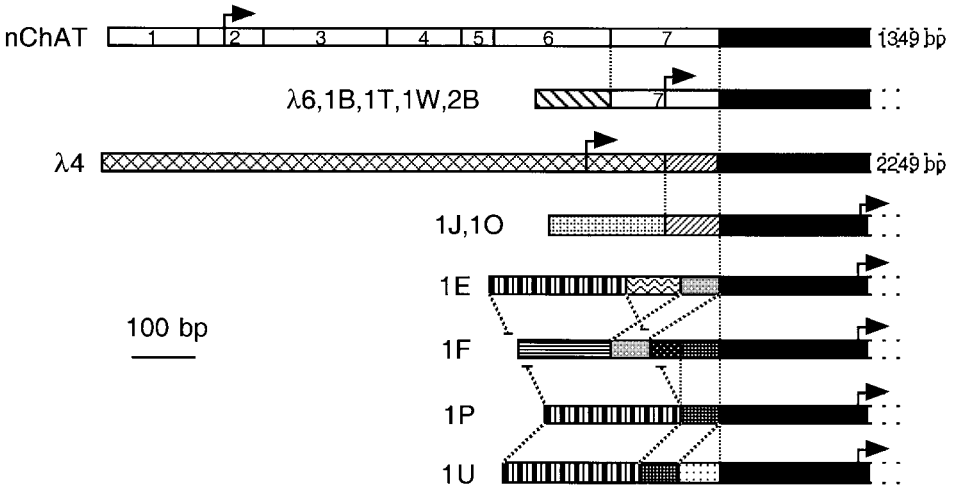

Fig. 2. Overview of the isolated testis-specific ChAT cDNA 5′-ends, and comparison to neuronal ChAT. Homologous sequences are denoted by bars filled with the same pattern, and are connected by dotted lines for clarification. The black bars denote exon 8 and downstream sequences, shared by all ChAT cDNAs. nChAT, neuronal ChAT; λ4, λ6, the two phage clones isolated from a rat testis cDNA library; 1B, 1E, 1F, 1J, 1O, 1P, 1T, 1U, 1W, and 2B denote 5′ RACE clones amplified from rat testis mRNA. Exons within nChAT (as described in Hahn et al., 1992) are shown, and the most 5′ ATGs surrounded by good Kozak sequences are indicated with arrows.

sequences in nChAT, λ4, and λ6 downstream from exon 8 (Fig. 2). None of these clones had a consensus translation initiation site within their respective unique upstream sequences.

Detection of Testis-Specific ChAT mRNAs by Ribonuclease Protection Assays

Probes were made from the testis-specific ChAT cDNA 5′ ends and used in RPA to confirm the presence of this mRNA species in vivo. A probe from positions 1 to 290 of clone λ6 was fully protected with testis but not with spinal cord mRNA (Fig. 4B). A probe from the 5′ end of λ4 also detected a testis-specific mRNA by RPA (data not shown). A probe corresponding to bp 899 to 1,187 of λ4, spanning the putative intron-exon boundaries was fully protected by an mRNA species in testis. A slightly smaller band, protected in testis mRNA, lacks 10 bases and probably corresponds to the mRNAs of type 1J/1O, that are colineal with clone λ4 downstream of position 909. The smallest band stems from protection of position 998 to 1,187, corresponding to exon 8 of nChAT, and the part shared by λ4 and λ6. This band, representing λ6 in the testis, was also detected in spinal cord and cortex mRNA (Fig. 4A), and confirms the restricted expression of nChAT mRNAs to neuronal tissues (Fig. 4B). Together, these data confirm the existence of novel ChAT mRNAs in testis with distinct 5′ flanking sequences, arising by a complex pattern of differential splicing.

Production of Truncated Testis-Specific ChAT Proteins by In Vitro Translation

The λ4 and λ6 as well as nChAT open reading frames were subcloned into the pcDNA3 expression vector (herein plasmids ptChATl4, ptChATl6, and pnChAT, respectively) and protein produced by combined in vitro transcription/translation. Polyacrylamide electrophoresis under denaturing conditions of ^{35}S-cysteine-labeled translation reactions revealed a product of approximately 35 kilodalton (kD) from the λ6 clone (Fig. 5A). nChAT produced a protein of the expected size at 60 kD, as well as minor amounts of a smaller protein, similar in size to the λ6 product, suggesting that full length neuronal ChAT mRNA may also give N-terminally truncated products similar to those found in testis. No band was visible from the translation of the λ4 cDNA

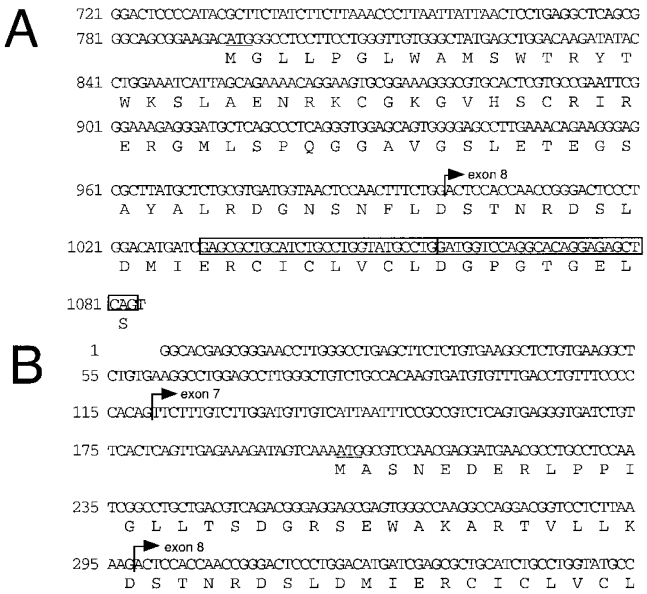

Fig. 3. Sequence surrounding the splice sites of the novel 5′ ends of testis-specific ChAT cDNAs to neuronal ChAT. (**A**) Clone λ4. (**B**) Clone λ6. Putative translation initiation sites are underlined. The homology to neuronal ChAT starts with exon 7 and exon 8 for clone λ6 and λ4, respectively. The two primers used for 5′ RACE are boxed in the λ4 sequence.

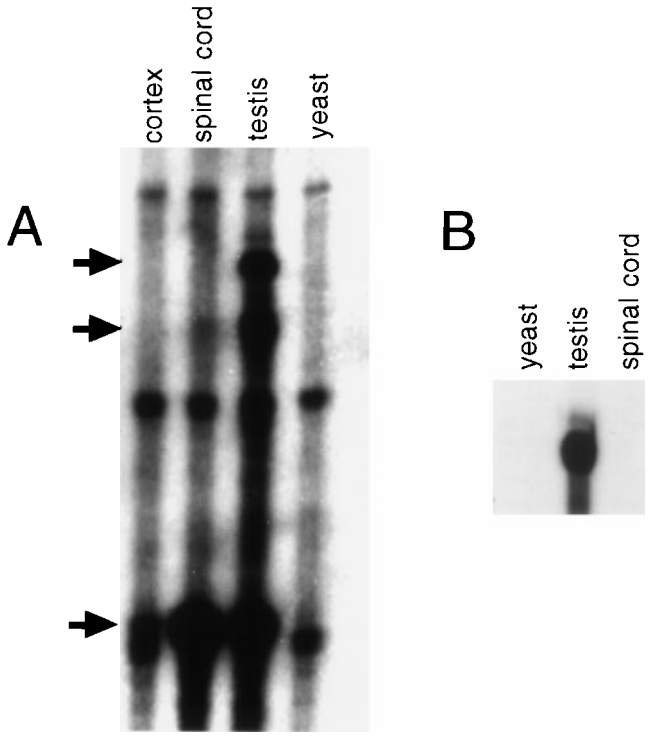

Fig. 4. Ribonuclease protection assays detecting testis-specific ChAT mRNAs. Five µg of polyadenylated mRNA from the indicated tissues were hybridized to ^{32}P-labeled RNA probes, and the protected products were separated with acrylamide gel electrophoresis. DNA sequence reactions of plasmids with known sequence were run in parallel to determine the sizes of protected bands (not shown). The probes used correspond to (**A**) basepair (bp) 899–1,187 of clone λ4, (**B**) bp 1–290 of clone λ6.

clone (Fig. 5A). This cDNA has an extended 5′ untranslated region, suggesting that this could have prevented efficient in vitro transcription from this clone. The putative open reading frame of λ4 clone was amplified by PCR, and at the same time, a hemagglutinin (HA) tag was added to the cDNA in the 3′ end, just upstream of the translation stop. Protein from the resulting construct was produced by in vitro translation, and a tagged product of the expected size (45 kDa) was detected with an anti-HA monoclonal antibody by western blot (Fig. 5B).

Truncated Testis-Specific ChAT Proteins Lack ChAT Activity

ChAT assays were performed with the in vitro translated proteins to assay their enzymatic activities. In vitro translated nChAT showed significant activity in this assay, demonstrating that the reticulocyte lysate allows for the synthesis of a biologically active enzyme. In vitro translated testis ChAT proteins, however, showed no ChAT activity (data not shown). We then tested whether any of the truncated proteins was able to bind either substrate of the ChAT reaction in a competitive assay with full length nChAT. For this purpose, pnChAT was co-transfected in COS cells with increasing amounts of either ptChATl6 (λ6 clone), ptChATl4HA (HA-tagged λ4 clone) or an empty pcDNA3 vector DNA. In all cases, equal amounts of total plasmid DNA were used for transfection. ChAT assays were performed with extracts from transfected cells in the presence of limiting amounts of either substrate, i.e., AcCoA or choline. Protein expression form the HA-tagged λ4 clone was confirmed by western blotting of transfected COS cell extracts using anti-hemagglutinin antibodies (data not shown). Neither of the testis-specific ChAT proteins reduced the activity of the neuronal ChAT enzyme in any of the tested conditions showing that the testis ChAT gene products do not bind ChAT substrates efficiently (Fig. 6).

DISCUSSION

The presence of the ChAT enzyme in testis has been debated for some time. Critics to the original proposal argued that carnitine acetyltransferase, which is very enriched in testis and can use choline as substrate, could account for the enzymatic activities detected. The molecular cloning of the ChAT gene in several mammalian species later established that testis produced ChAT mRNA, albeit of a reduced size compared to that found in brain and spinal cord (Ibáñez et al., 1991). A polyclonal antiserum was also used to demonstrate ChAT-like immunoreactivity in the sperm (Ibáñez et al., 1991). The evidence marshaled in by these molecular studies dissipated the initial controversy and established the testis as a major site of ChAT expression outside the nervous system. Here we show that, while several ChAT mRNAs are produced in the testis, these are incapable of coding for a full-length, active ChAT enzyme. Thus, the nervous system remains so far the only major site of bona-fide ChAT synthesis in mammals.

Unique, tissue-specific mRNAs are produced from the ChAT gene in rat testis. They differ from the neuronal mRNAs in the 5′ end, and have the potential of producing either truncated proteins or proteins with a novel N-terminal sequence. The testis-specific 5′ sequences are spliced to either exon 7 or 8 of nChAT; from this point, the novel clones are colineal to neuronal ChAT.

The presence of special testicular isozymes is not unique to the ChAT protein. A very similar situation is found in the angiotensin-converting enzyme (ACE) gene (Howard et al., 1990). Here, a testis-specific first exon is located in the intron between exons 12 and 13 of the somatic ACE gene. This first exon splices to somatic exon 13, and from this point onwards the sequences are identical. This results in a truncated testis ACE of 732 amino acids of which the first 66 originate from the testis-specific exon. Interestingly, the somatic ACE protein has been shown to consist of two homologous domains, each coded by 12 exons. Testis ACE contains only the C-terminal domain, but retains enzymatic activity (Howard et al., 1990). ACE knockout mice lacking both isozymes exhibit low blood pressure, kidney dysfunctions, and male infertility (Krege et al.,

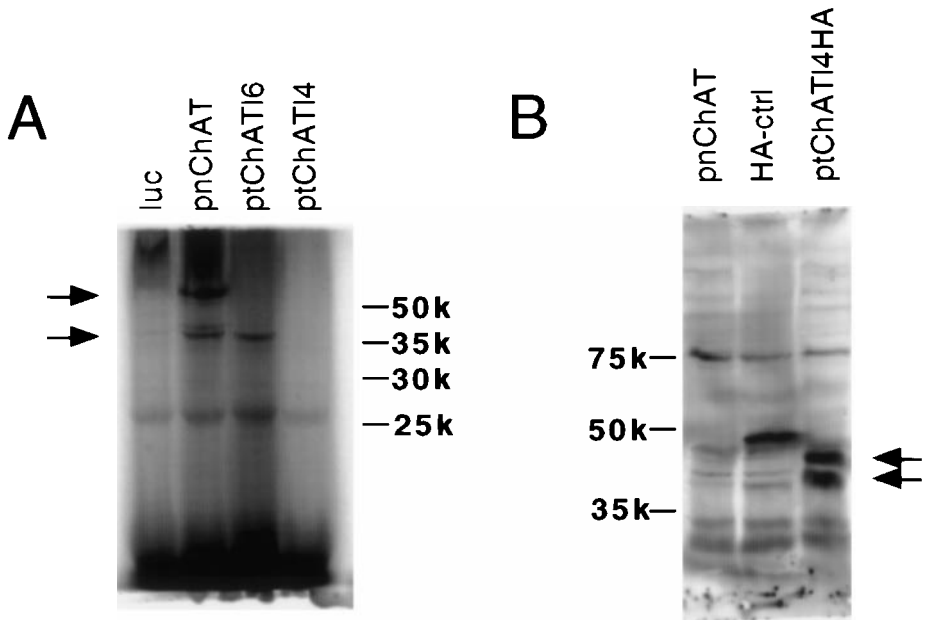

Fig. 5. In vitro translation of the testis-specific ChAT cDNAs. (**A**) The indicated proteins were produced by in vitro translation in a rabbit reticulocyte lysate in the presence of ^{35}S-metionine, and run on a 10% denaturing polyacrylamide gel and exposed to phopshoscreens. luc, luciferase control plasmid; pnChAT, neuronal ChAT; ptChATl6, testis ChAT cDNA clone λ6; ptChATl4, testis ChAT cDNA clone λ4. The position of molecular size markers run in a parallel lane of the gel is indicated in kD. (**B**) Western blot of in vitro translated products. After running translation reactions on a 10% denaturing polyacrylamide gel, the proteins were blotted onto a nitrocellulose filter, and detected with a monoclonal antibody against the HA-epitope. luc, luciferase control plasmid; pnChAT, neuronal ChAT; HA-ctrl, positive control HA-tagged 48 kDa protein; ptChATl4HA, HA-tagged testis ChAT cDNA clone λ4. The position of molecular size markers run in a parallel lane of the gel is indicated in kD.

1995). Selective restoration of expression of the testicular isozyme in sperm cells completely rescued the fertility deficits of male mice but had no effect on blood pressure or kidney dysfunction (Ramaraj et al., 1998), demonstrating that the role of ACE in male fertility is completely dependent on the expression of the testicular isoform in sperm. Another example is the human gene encoding neuronal nitric-oxide synthase (nNOS), which is also expressed in the testis (Wang et al., 1997). The testis has three unique non-coding 5′ exons, denoted T1b, T1, and T2, contained within intron 3 of the neuronal gene. These are spliced to a common exon 4 (E4) of the neuronal gene either as T1-T2-E4, T1b-T2-E4, or T1-E4. The predicted ATG of these mRNAs is situated within exon 5 of the full nNOS gene, thereby producing a protein with a truncated N-terminus missing 336 aa of the neuronal nNOS sequence. Despite this truncation, the protein, which is unique to the testis, shows enzymatic activity comparable to full-length nNOS (Wang et al., 1997). Again, a similar situation is seen in the mouse gene for adenosine deaminase (Meng et al., 1997). The testis-specific mRNA version of this gene lacks the first two exons, and the putative ATG in the third exon produces a protein lacking the first 51 amino acids. The testis also expresses the somatic mRNA, though this transcript is differently regulated during development (Meng et al., 1997). The enzymatic activity of the testis-specific gene product of adenosine deaminase was not investigated in this study.

These studies, and our results, suggest that several different genes, including several coding for peptide hormones and enzymes, have alternative promoters in testis that direct the synthesis of novel mRNAs encoding N-terminally truncated protein products. An obvious advantage of having a separate promoter for the testis ChAT gene is that it may allow for a regulatory regime distinct from that of nChAT. In spinal cord, for example, ChAT mRNA reaches adult levels around the second week postnatally, whereas in the testis a peak is observed at 28 days, after which levels decrease (Ibáñez et al., 1991).

The presence of different forms of ChAT enzyme in testis might also reflect the subcellular location. Immunoreactivity to testis ChAT is found both in the post-acrosomal region of the spermatozoa head, and also as a narrow band in the midpiece of the tail (Ibáñez et al., 1991). Perhaps the unique N-terminal sequence of testicular ChAT is important for translocation of the protein to the proper compartment in the sperm cell. Alternatively, the amino acid residues unique to testis ChAT may be involved in the regulation or function of the protein. An intriguing feature of all products of the ChAT gene made in testis is that they do not display ChAT activity, nor do they appear to bind either ChAT substrate in a competition assay.

Several studies have investigated structure-function relationships in ChAT. Site-directed mutagenesis has been used to localize catalytically important histidines,

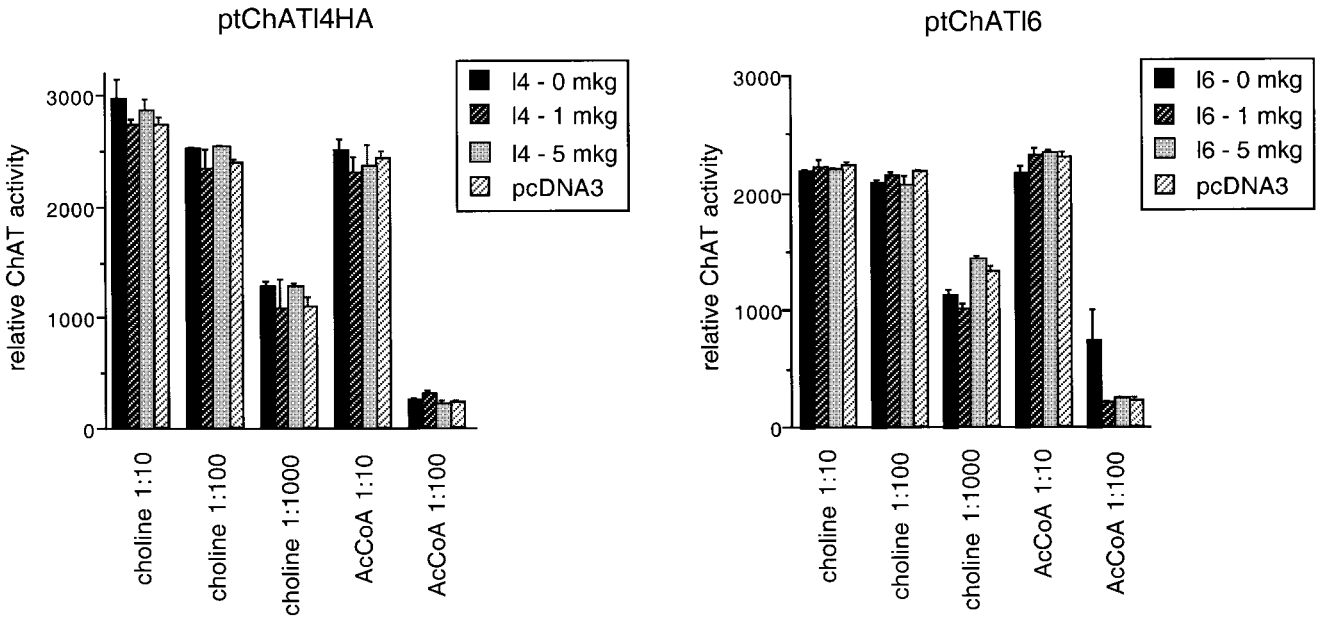

Fig. 6. ChAT assays on extracts from COS cells co-transfected with varying amounts of testis-specific ChAT cDNAs and a neuronal ChAT cDNA. 0,1, and 5 μg of either ptChATl4HA or ptChATl6 were transiently co-transfected with 1 μg of pnChAT in COS cells. An expression plasmid producing a non-functional protein was added to keep total DNA amounts constant. As an additional negative control, 5 μg of empty expression plasmid pcDNA3 was substituted for the testis ChAT cDNA expressing plasmids in one sample. Cells were harvested after 48 hr, and ChAT assays were performed with varying amounts of either choline or acetyl coenzyme A (AcCoA). These substrates were diluted 10, 100, or 1,000 times compared to the standard assay as indicated.

in particular, His-426 was found crucial for enzyme activity (Carbini and Hersch, 1993). Another study comparing the sequences of several ChAT proteins found a highly conserved sequence RRLRW/YKC located around position 380 of nChAT. This region is homologous to one found in arylamine N-acetyltransferases, and is likely part of the catalytic domain (Ishii et al., 1990). Both this region and the His-426 are found within the sequence common to testis and neuronal ChAT. In a study by Finocchiaro et al., a putative choline binding domain was proposed based on sequence homology among acyltransferases (Finocchiaro et al., 1991). A highly conserved leucine-proline motif was found in ChAT of four different species as well as in several carnitine acyltransferases, but was not present in enzymes interacting with CoA derivatives, thus pointing to this motif as being important for binding of the chemically related compounds choline and carnitine (Finocchiaro et al., 1991). This domain, EELDLPKLPVPPLQ, comprises amino acid positions 19–32 of rat nChAT, and it is absent in the testis-specific forms of ChAT, which could potentially serve as an explanation for their lack of ChAT activity.

The function of testis-specific ChAT-related proteins remains unknown. Further studies on the structure and function of domains in the ChAT protein should help to shed light on this problem.

CONCLUSIONS

In this study we show that the previously reported ChAT gene product present in testis is derived from several testis-specific ChAT mRNAs that contain novel 5′ sequences. One of these codes for a putative protein in which the first 262 amino acids of neuronal-type ChAT is replaced by 69 testis-specific amino acids. The others code for N-terminally truncated proteins. The testicular ChAT proteins were produced in vitro and in transfected cells and were found to lack enzymatic activity in ChAT assays. In addition, they did not bind either of the ChAT enzyme substrates as determined in a competition assay. Thus, these ChAT-like proteins most likely serve a unique function in testis.

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