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Expression of choline acetyltransferase mRNA in spermatogenic cells results in an accumulation of the enzyme in the postacrosomal region of mature spermatozoa

(acetylcholine/testis/Northern blot analysis/*in situ* hybridization/immunohistochemistry)

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ABSTRACT **The gene encoding choline acetyltransferase (ChAT; EC 2.3.1.6), the key enzyme in the synthesis of the neurotransmitter acetylcholine (ACh), is shown to be expressed in rat and human testes. High levels of two ChAT transcripts of 3.5 and 1.3 kilobases were detected by Northern blot analysis of adult rat testis RNA. A single ChAT mRNA species of 3.2 kilobases was detected in human testis. Cells responsible for the synthesis of ChAT mRNA in rat testis were localized by *in situ* hybridization in the middle part of the seminiferous epithelium, where the labeling was mostly found over spermatocytes and spermatids. Studies on the ontogeny of ChAT mRNA expression showed low levels in prepubertal rats with increasing levels as sexual maturation is reached. A peak of expression was seen at postnatal day 32, correlating with the onset of postmeiotic spermatogenesis. Results from surgical and pharmacological treatments suggest that androgens, as well as pituitary factors, could influence the relative levels of the two ChAT mRNAs detected in rat testis. Evidence for translation of the mRNA detected in the testis was obtained from the demonstration of ChAT-like immunoreactivity in ejaculated human spermatozoa. The staining was restricted to the postacrosomal region of the head, where the membrane of the sperm first fuses with that of the egg during fertilization, and to the annulus, a ring of dense material in the caudal end of the midpiece. Combined, these findings support the hypothesis that the neurotransmitter ACh is involved in reproductive function.**

Neurotransmitter-synthesizing enzymes are mainly expressed in the nervous system, where their enzymatic products function in synaptic transmission. An increasing number of neuropeptides, which may function as neuromodulators/neurotransmitters, has also been detected in the nervous system. However, in recent years a number of neurotransmitters and neuropeptides have in addition been shown to be synthesized in nonneuronal tissues, suggesting a broader function for these products than previously postulated (1). In particular, several different neuropeptides have been shown to be expressed in reproductive tissues. Proopiomelanocortin-derived peptides have been detected in the testis (2, 3). The two other opioid peptide precursors, preproenkephalin and prodynorphin (4, 5), and the corticotropin-releasing factor (6, 7) have also been demonstrated in reproductive tissues from different species. The gene encoding the neurotransmitter/neuromodulator cholecystokinin (CCK) has been shown to be expressed in male germ cells of several species, with an accumulation of the peptide in the acrosomal granule (8). In man, similar observations have been made for the CCK-related hormone gastrin (9). The results of these studies suggest that CCK peptides, and gastrin peptides in man, can be released during the acrosome reaction and therefore may be one of the components that activate the oocyte for fertilization. Recently, mRNA encoding glutamic acid decarboxylase (GAD), the key enzyme in the synthesis of the inhibitory neurotransmitter γ-aminobutyric acid (GABA), was shown to be expressed in male germ cells of several species (10). GAD-like immunoreactivity was detected in the midpiece of ejaculated human spermatozoa, where mitochondria that power the flagellum are located, suggesting that GABA may be important for sperm motility.

Acetylcholine (ACh) is a low molecular weight neurotransmitter produced by the enzyme choline acetyltransferase (ChAT; EC 2.3.1.6) and present in central and peripheral neurons. Several reports have suggested that the ACh cycle (synthesis, stimulation at a receptor, and hydrolysis by cholinesterase) also plays a significant role in nonneuronal tissues, including spermatozoa (see ref. 11 for a review). ACh has been demonstrated in the cytoplasm of spermatozoa of a number of mammalian species (12, 13). An acetylcholinesterase (AChE)-like enzyme has been found in mammalian spermatozoa, where cytochemical data indicated AChE located underneath the plasma membrane (14–17). Also, several studies using cholinergic agonists and antagonists have provided evidence that spermatozoa contain a cholinergic receptor of nicotinic type (11, 18–20). Finally, the presence of ChAT in mammalian spermatozoa has been inferred based on the detection of ChAT-like activity in sperm extracts and in different spermatozoal fractions (12, 17, 21, 22). However, these studies have been reevaluated in view of the possibility that the activity was mistaken for carnithine acetyltransferase (23, 24), which is present in high amounts in rat epididymal tissues and spermatozoa (25), and can also utilize choline as a substrate (26).

In the present study, ChAT mRNA was demonstrated in rat and human testes. ChAT mRNA expression was localized to spermatogenic cells by *in situ* hybridization, and the ontogeny of ChAT mRNA expression and its hormonal regulation were studied by Northern blot analysis. The demonstration of ChAT-like immunoreactivity (ChAT-LI) in ejaculated human spermatozoa strongly suggests that the ChAT mRNA detected in spermatogenic cells causes an endogenous production of ChAT and ACh, which may play a physiological role in the male reproductive system.

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Abbreviations: ACh, acetylcholine; ChAT, choline acetyltransferase; ChAT-LI, ChAT-like immunoreactivity; EDS, ethylene dimethanesulfonate; ABP, androgen-binding protein; GAD, glutamic acid decarboxylase; AChE, acetylcholinesterase.

MATERIALS AND METHODS

Pharmacological Treatments and Lesions. Testes from x-irradiated rats were prepared essentially according to the method of Beaumont (27). Pregnant rats were irradiated with 1.5 Gy as a single dose (0.011 Gy per sec; focal length, 70 cm) at day 19 of gestation. Testes obtained from the 90-day-old male offspring and from age-matched controls were kept frozen at −70°C until use. Testicular weights were 0.44 g per testis in irradiated rats and 2.12 g per testis in control rats. Specific destruction of Leydig cells was performed by a single s.c. injection of ethylene dimethanesulfonate [EDS, 75 mg/kg of body weight (EDS was kindly provided by Anders Bergh, University of Umeå, Sweden)] (28). Testes were removed 6 days after the treatment. Testosterone concentrations measured by radioimmunoassay in testicular tissue homogenates were 96 ng/g of tissue in intact and 1.3 ng/g in EDS-treated testes. Hypophysectomy of adult rats was performed transauricularly (29). Testis weight decreased 10-fold 2 weeks after hypophysectomy. Testosterone substitution of hypophysectomized rats was performed by daily s.c. injections of 10 mg of testosterone propionate in sesame oil for 2 weeks, starting 1 day after hypophysectomy. In order to block androgen action, 55-day-old Sprague–Dawley rats were given daily s.c. injections of 30 mg of the antiandrogen cyproterone acetate (Schering) (30). The testes were removed 14 days after continuous treatment and kept frozen at −70°C until use (testicular weights were 1.02 g per testis in treated rats and 1.78 g in control rats).

RNA Preparation and Northern Blot Analysis. Poly(A) RNA was prepared from homogenized tissue samples as described (31). Twenty-five micrograms of Poly(A) RNA from each sample was electrophoresed in 1% agarose gels containing 0.7% formaldehyde and transferred to nitrocellulose filters. The filters were then hybridized to a rat ChAT cDNA probe comprising the entire protein coding sequence of the rat ChAT gene [positions 202–2133 in the published sequence of Brice *et al.* (32)] isolated by polymerase chain reaction amplification from rat spinal cord cDNA as described (33). Where indicated, a 735-base-pair (bp) androgen-binding protein (ABP) cDNA probe (34) was also used. Probes were labeled with [α-^{32}P]dCTP by nick-translation to a specific activity of $\approx 5 \times 10^8$ cpm/μg. Hybridization was performed in 4× SSC (1 × SSC is 150 mM NaCl/15 mM sodium citrate, pH 7.0), 40% formamide, 1× Denhart's solution, and 10% dextran sulfate at 42°C. Filters were washed at high stringency (0.1× SSC/0.1% SDS, 60°C) or, where indicated, low stringency (0.05× SSC/0.1% SDS, 45°C) and exposed to Kodak XAR-5 films.

***In Situ* Hybridization.** Rat testis sections (14 μm) were hybridized to a 43-mer oligonucleotide complementary to rat ChAT mRNA [positions 1818–1860 in the rat ChAT cDNA (32)] as described (35). Adjacent sections were hybridized to a similar length and G + C content oligonucleotide complementary to rat calcitonin gene-related peptide mRNA (36) as control.

Immunohistochemistry. Ejaculated human spermatozoa were obtained from healthy volunteers. Cells were smeared onto gelatin-coated glass slides, dried, and fixed as described (10). Slides were incubated with a polyclonal goat antiserum against human ChAT (37) diluted 1:400. Primary antibody was visualized with fluorescein-labeled donkey anti-goat IgG (Nordic, Tilburg, The Netherlands). Normal goat serum was used as control. Sections were examined in a Nikon Microphot-FX fluorescence microscope.

RESULTS

Expression of ChAT mRNA in Rat and Human Testes. Analysis of poly(A) RNA from 10 different adult rat organs by Northern blot analysis using a rat ChAT probe comprising the entire protein coding sequence of the ChAT gene showed, as expected (32, 33, 38), high levels of a 4.0-kilobase (kb) ChAT mRNA in the rat brain (Fig. 1*A*). In addition, two transcripts of about 3.5 and 1.3 kb were detected in the testis. The level of ChAT mRNA in the testis was comparable to the level found in brain. The amount of ChAT mRNA was below detection limits in all other organs tested. The rat ChAT probe also detected, at lower stringency, a transcript of 3.2 kb in human testis (Fig. 1*B*). In contrast to the rat, no small ChAT transcript could be detected in the human testis.

Since the open reading frame encoding an active ChAT protein comprises 1932 nucleotides (32), the 1.3-kb transcript detected in rat testis is clearly nonfunctional as far as encoding ChAT. In an attempt to further characterize the ChAT transcripts detected in rat testis, consecutive restriction fragments from a 1.93-kb rat ChAT cDNA clone (33) were used as probes for Northern blot analysis. Whereas all probes hybridized with the 3.5-kb transcript, only the two most 5′ fragments (*Eco*RI–*Ava* I, 570 bp, and *Ava* I–*Sac* I, 298 bp) detected the 1.3-kb mRNA (Fig. 1*C*). However, both transcripts could be detected when an 850-bp *Pst* I rat genomic fragment containing 5′ untranslated sequences of the rat ChAT gene was used as probe (Fig. 1*C*). These results suggest tat both transcripts have the same 5′ end but differ at their 3′ ends.

Localization of ChAT mRNA-Expressing Cells in Rat Testis by *in Situ* Hybridization. A 43-mer oligonucleotide complementary to rat ChAT mRNA extending from position 1818 to 1860 in the rat ChAT cDNA (32) was used as a probe for *in situ* hybridization. This probe is included in the *Ava* I–*Ava* I 164-bp cDNA fragment shown in Fig. 1*C* and will only detect cells expressing the 3.5-kb transcript. In rat testis sections, labeling for ChAT mRNA was found in ≈40% of the seminiferous tubules (Fig. 2*A*), where it was localized to the middle part of the seminiferous epithelium (Fig. 2*B*). At higher magnification, labeling was observed mainly over spermatocytes and spermatids (Fig. 2*C*), whereas Sertoli

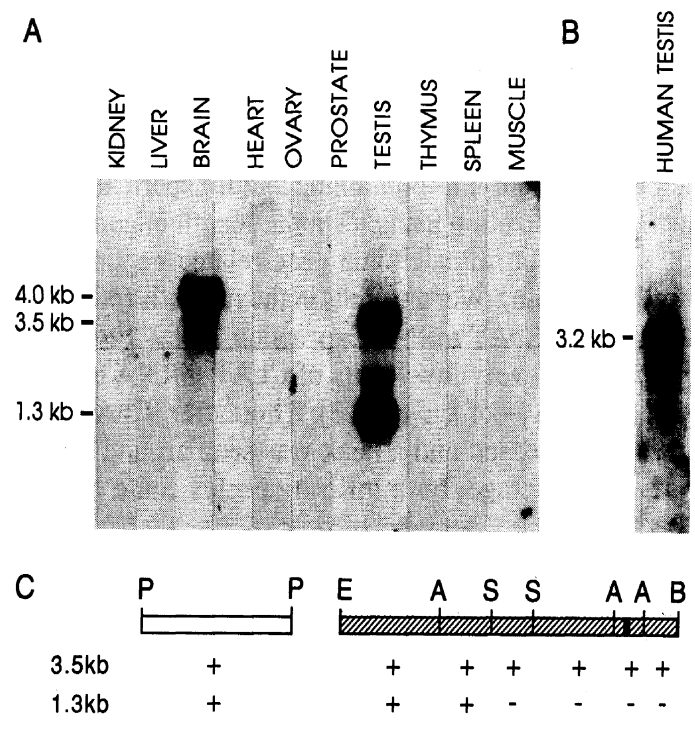

FIG. 1. Expression of ChAT mRNA in rat and human testes. (*A*) Poly(A) RNA from the indicated adult rat organs was hybridized to a 1.93-kb rat ChAT cDNA probe. (*B*) Same analysis as in *A* using poly(A) RNA from human testis. (*C*) Schematic representation of the results from hybridization of rat testis RNA to consecutive restriction fragments of the 1.93-kb ChAT cDNA probe (hatched box) or to an 850-bp genomic fragment from the 5′ end of the rat ChAT gene (open box). A + or − sign denotes whether the indicated ChAT transcript hybridized or not with the corresponding probe. The small filled box shows the location of the oligonucleotide probe used for *in situ* hybridization. A, *Ava* I; B, *Bam*HI; E, *Eco*RI; P, *Pst* I; S, *Sac* I.

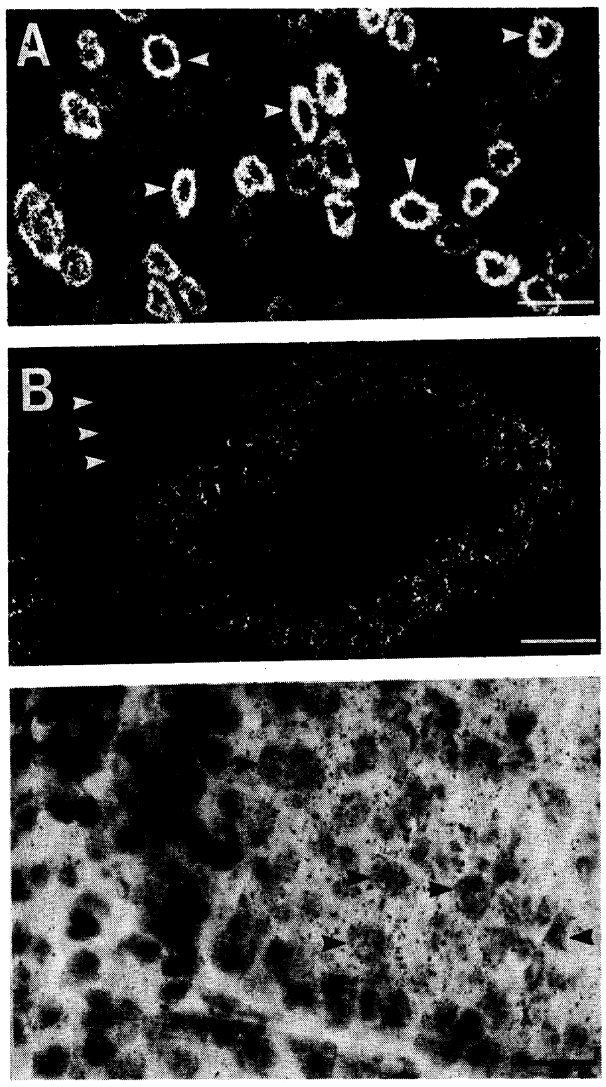

FIG. 2. Localization of ChAT mRNA-expressing cells in rat testis by *in situ* hybridization. (*A*) X-ray film autoradiograph of a rat testis section after hybridization with a rat ChAT oligonucleotide probe. Note intense labeling over the seminiferous tubules (arrowheads). (Bar = 500 μm.) (*B*) Emulsion autoradiograph showing ChAT mRNA labeling over the middle part of the seminiferous epithelium. Interstitial tissue (arrowheads) and lumen are devoid of labeling. (Bar = 40 μm.) (*C*) High-magnification, bright-field micrograph of rat seminiferous epithelium. Note labeling over spermatocytes and spermatids (arrowheads). (Bar = 25 μm.)

cells, spermatogonia, and interstitial tissue were devoid of labeling altogether. No labeling was found either over the central parts of the seminiferous tubules (Fig. 2*B*) or over the lumen of the epididymis containing mature spermatozoa (not shown). Hybridization using a similar length oligonucleotide probe specific for rat calcitonin gene-related peptide mRNA showed no labeling over any cells in the rat testis (not shown).

Ontogeny of ChAT mRNA Expression in Rat Testis. In the prepubertal rat, very low levels of ChAT mRNA could first be seen at 24 days of age (Fig. 3). Thereafter, ChAT mRNA expression increased and a peak was seen around postnatal day 32. At this stage, both mRNA species could be clearly detected, although the larger transcript was present at higher levels. After postnatal day 32, ChAT mRNA levels decreased and the relative concentration of ChAT mRNA in the adult testis was ≈7 times lower than that in the 32-day-old testis. However, in contrast to the immature testis, the adult testis contained a larger proportion of the smaller 1.3-kb transcript.

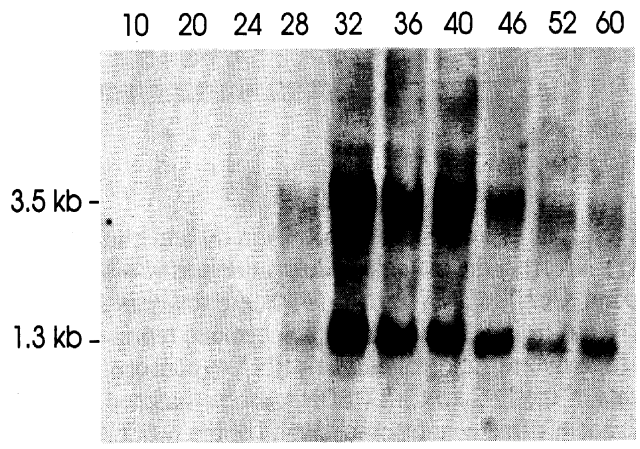

FIG. 3. Ontogeny of ChAT mRNA expression in the rat testis. Poly(A) RNA from rat testes of the indicated ages (postnatal day) was hybridized to a 1.93-kb rat ChAT cDNA probe.

Hormonal Regulation of ChAT mRNA Expression in Rat Testis. As a first step toward an understanding of the regulation of ChAT mRNA expression in the rat testis, the effects of pharmacological treatments and lesions, known to affect testicular function, were examined. Levels of ChAT mRNA were analyzed in rat testis after the Leydig cells had been specifically destroyed by a single injection of EDS, resulting in an ≈30-fold decrease in the level of testosterone in the testis 6 days after the injection. This treatment decreased by about 50% the level of the 3.5-kb ChAT transcript, although it had no effect on the 1.3-kb mRNA (Fig. 4*A*). Expression of ChAT mRNA was also studied in the testis of 90-day-old rats that had been x-irradiated *in utero* at gestation day 19. The x-ray dose was titrated so that it preferentially destroyed germ-cell precursors, resulting in a 95–98% decrease in the number of germ cells at puberty. This treatment resulted in a dramatic drop in the levels of ChAT mRNA (Fig. 4*A*). In contrast, the mRNA levels for the Sertoli cell-specific product ABP were unchanged or slightly increased in the testes of EDS- or x-ray-treated rats, respectively (Fig. 4*B*), indicating that Sertoli cell survival was not affected by either of these lesions.

Levels of ChAT mRNA were also analyzed in testes from adult rats 2 weeks after hypophysectomy. Interestingly, the treatment significantly increased the relative amount of the 3.5-kb transcript, whereas the level of the 1.3-kb mRNA was reduced compared to control animals (Fig. 4*C*). This effect could be suppressed by testosterone substitution of hypophysectomized animals (Fig. 4*C*). In contrast to hypophysectomy, treatment with cyproterone acetate, which specifically blocks androgen receptors but no other steroid receptors (30), significantly decreased the level of the 3.5-kb transcript, whereas the amount of the 1.3-kb mRNA was increased (Fig. 4*C*).

Immunohistochemical Localization of ChAT-LI in Ejaculated Human Spermatozoa. Immunohistochemical staining of ejaculated human spermatozoa with a ChAT-specific antiserum revealed intense immunoreactivity in the head of the spermatozoa, usually as a double band encircling the post-

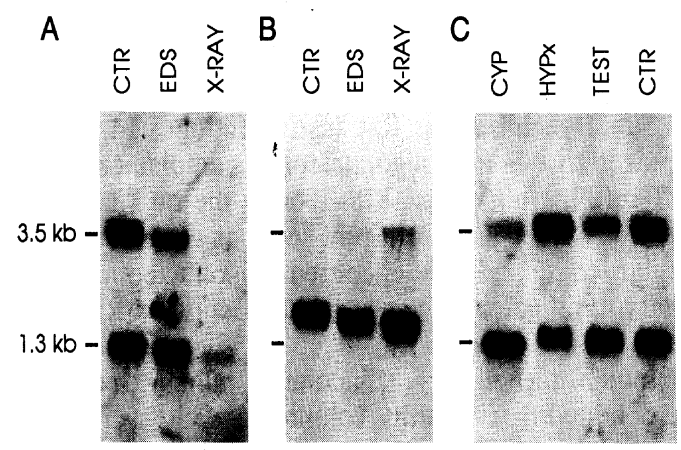

FIG. 4. Effects of surgical and pharmacological treatments on the level of ChAT mRNA in rat testis. (*A*) Poly(A) RNA from testes of 90-day-old rats that had been x-irradiated *in utero* at gestational day 19 (X-RAY), adult rats that had been treated with EDS, or age-matched controls (CTR) was hybridized to a 1.93-kb rat ChAT cDNA probe. (*B*) Same analysis as in *A* using a 735-bp rat ABP cDNA probe (34). (*C*) Poly(A) RNA from testes of rats that had been treated for 2 weeks with the anti-androgen cyproterone acetate (CYP), had been hypophysectomized 2 weeks earlier (HYPx), or had been hypophysectomized and then substituted with testosterone for 2 weeks (TEST) or age-matched controls (CTR) was hybridized to a 1.93-kb rat ChAT cDNA probe.

acrosomal region (Fig. 5). An intense thin band across the proximal region of the tail was also detected in most spermatozoa (Fig. 5*B*). No staining was observed in any other part of the cell (Fig. 5) or in controls incubated with a normal goat serum and secondary antibodies (not shown).

DISCUSSION

In this study, expression of mRNA encoding ChAT, the key enzyme in the synthesis of the neurotransmitter ACh, was demonstrated in rat and human testes by Northern blot analysis. Several lines of evidence, including data from *in situ* hybridization, suggest that the ChAT mRNA detected in testis is derived from germ cells and that its expression is developmentally as well as hormonally regulated.

High levels of two ChAT mRNAs of about 3.5 and 1.3 kb were detected in adult rat testis. Northern blot analysis of rat testis RNA using consecutive restriction fragments comprising the entire protein coding sequence of the ChAT cDNA showed that both transcripts probably have the same 5′ ends but the small transcript only contains the first 800 nucleotides of coding sequence and can therefore not encode a functional ChAT protein. Although the 3.5-kb mRNA was 500 bases shorter than the one found in brain, the fact that it hybridized with all of the ChAT cDNA fragments tested strongly suggests that it contains all of the necessary sequences to encode an active ChAT protein. Several other neurotransmitter-synthesizing enzymes and neuropeptides expressed in testis also show different mRNA sizes from their respective transcripts in the brain. In the case of proopiomelanocortin, the predominating transcript in testis is 250 nucleotides shorter than that in the pituitary (39). Recently, a GAD mRNA species of about 2.5 kb was detected in rat testis, 1.2 kb smaller than the species found in brain (10). The size differences between mRNAs from testis and their corresponding transcripts in brain could be the result of a number of mechanisms, including transcription initiation, RNA processing, or polyadenylylation. As germ-cell differentiation proceeds into spermiogenesis, a number of chromatin changes take place coinciding with the termination of RNA synthesis (40, 41). The transcriptional inactivation of the spermatid nucleus during spermiogenesis requires that a late expressed gene must be translated from a stored mRNA, and several studies have indicated that RNAs synthesized during germ-cell maturation have a long lifetime (40, 42). Thus, the size differences observed between some mRNAs from testis and other sources might be the result of the elimination of destabilizing sequences in order to increase the stability of a message to be translated during late stages of germ-cell development. Sequences responsible for destabilization of the corresponding mRNA have been identified, for example, in the 5′ and 3′ untranslated regions of c-*myc* mRNA (43–45).

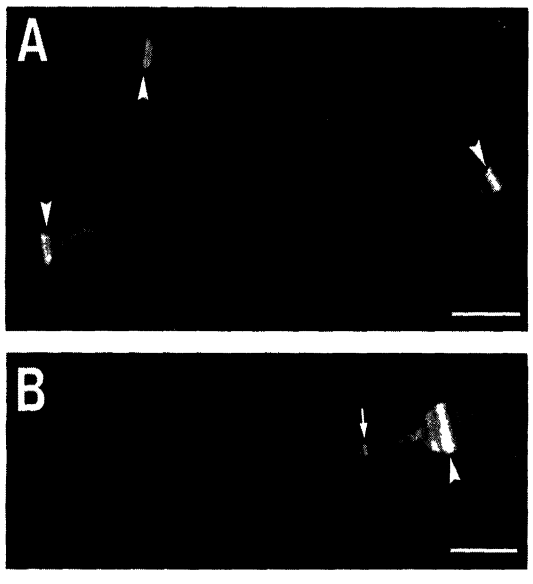

FIG. 5. Immunohistochemical localization of ChAT-LI in ejaculated human spermatozoa. (*A*) Intense immunofluorescence can be seen in the postacrosomal region of the head (arrowheads). (Bar = 20 μm.) (*B*) At higher magnification the staining appears as a double band around the postacrosomal region (arrowhead). A thin band of ChAT-LI is also present in the proximal region of the tail (arrow), at the approximate location of the annulus. (Bar = 10 μm.)

The low level of ChAT mRNA observed after destruction of germ-cell precursors by *in utero* x-irradiation emphasized the importance of normally developing germ cells for ChAT mRNA expression in rat testis. In agreement with this result, *in situ* hybridization revealed ChAT-mRNA-positive cells in the middle part of the seminiferous tubules where developing germ cells are located. The labeling was confined to spermatocytes and spermatids with no labeling over spermatogonia, Sertoli cells, or interstitial tissue. The analysis of the ontogeny of ChAT mRNA expression in rat testis did not show detectable levels until 24 days of age, with a peak around 32 days and a subsequent decline in the adult. Hence, expression of ChAT mRNA in rat testis appears to coincide with the start of postmeiotic spermatogenesis, which, in the rat, occurs at about 25 days of age (46). The drop after postnatal day 40 could be caused by a dilution of the spermatogenic cells responsible for ChAT mRNA expression due to the appearance of other cell types such as spermatozoa.

It is well established that sperm production requires the stimulatory actions of the anterior pituitary gonadotropins, luteinizing hormone (LH), and follicle-stimulating hormone (FSH). Stimulation of testosterone synthesis in Leydig cells by LH is important for the initiation and maintenance of spermatogenesis, and FSH binds to receptors on Sertoli cells and stimulates Sertoli cell production of a variety of proteins (e.g., ABP) that play an important role in spermatogenesis (47, 48). In rat testis, hypophysectomy significantly increased the relative ratio of the 3.5-kb ChAT mRNA to the 1.3-kb ChAT mRNA 2 weeks after the lesion, suggesting that pituitary-derived products might be involved in the regulation of ChAT mRNA in rat testis. On the other hand, since hypophysectomy also alters the relative abundance of the different cell types in the testis, the effect of the lesion on the ChAT mRNA pattern could also be caused by a specific destruction of testicular cell types expressing the 1.3-kb transcript. Interestingly, the pattern of ChAT mRNA expression after hypophysectomy resembles the one seen in the prepubertal rat, at the time where the first spermatocytes and spermatids are detected. The fact that the effects of hypophysectomy could be suppressed by testosterone substitution indicates that this hormone might be involved in the regulation of ChAT mRNA expression in the testis. Further evidence for a role of testosterone in the regulation of ChAT mRNA expression in testis was obtained after destruction of Leydig cells and blocking of androgen receptors. However, in this case, both treatments decreased the ratio between the 3.5-kb to 1.3-kb transcripts. These results suggest that androgens, as well as pituitary factors, can influence the level of ChAT mRNA in rat testis.

Several previous studies have indicated a role for ACh in a number of processes with regard to testicular and reproductive functions. Kasson and Hsueh (49) have shown that nicotinic cholinergic agonists suppressed human chorionic gonadotropin-stimulated androgen production in primary cultures of rat testicular cells, suggesting the presence of nicotinic cholinergic receptors on Leydig cells. It has also been proposed that a local ACh cycle might be involved in the coordination of contraction and relaxation cycles responsible for the motility of spermatozoa (12, 22). In agreement with this possibility, a number of cholinergic agonists and antagonists (19, 20), AChE inhibitors (50), and ChAT inhibitors (22) have been demonstrated to affect sperm motility. Ovarian and mature mouse oocytes (51) and human ovarian

oocytes (52) respond to low concentrations of ACh by hyperpolarizing the membrane and concomitantly increasing input resistance in a dose-dependent manner. It was suggested that ACh from the sperm might be involved in activation processes triggered by sperm–egg interaction.

Direct evidence for the presence of ChAT protein in spermatogenic cells was obtained from the demonstration of ChAT-LI in ejaculated human spermatozoa. Interestingly, the labeling was not evenly distributed but concentrated as a double band around the postacrosomal region of the head, possibly in the equatorial segment. Caudal to the acrosome, the spermatozoa from several mammals have a specialized dense layer located between the plasma membrane and the nuclear envelope. This layer, which appears as a broad band encircling the postacrosomal region, is believed to be an important structure, since it constitutes the region of the sperm membrane that first fuses with that of the egg during fertilization. In most cells, a thin band of immunoreactivity encircling the proximal region of the tail was also detected, in the approximate location of the annulus, a ring of dense material fused to the plasma membrane at the junction of the midpiece and principal piece of the tail. This finding is intriguing in view of the proposed role of ACh in sperm motility. Hence, the localization of ChAT-LI in the spermatozoon suggests that the ACh endogenously produced in these cells could be involved in sperm motility as well as in the fertilization process.

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1. Persson, H., Ericsson, A., Brené, S., Ooi, M., McIntyre, K. R., Hökfelt, T., Pelto-Huikko, M., Rehfeld, J. & Schalling, M. (1990) in *Neuropeptides and Their Receptors*, Alfred Benzon Symposium 29, eds. Schwartz, T., Hilstead, L. & Rehfeld, J. (Munksgaard, Copenhagen), pp. 106–116.
2. Tsong, S. D., Phillips, D., Halmi, N., Liotta, A. S., Margioris, A., Bardin, C. W. & Krieger, D. T. (1982) *Endocrinology* **110,** 2204–2206.
3. Margioris, A. N., Liotta, A. S., Vaudry, H., Bardin, C. W. & Krieger, D. T. (1983) *Endocrinology* **113,** 663–671.
4. Kilpatrick, D. L. & Rosenthal, J. L. (1986) *Endocrinology* **119,** 370–374.
5. Douglass, J., Cox, B., Quinn, B., Civelli, O. & Herbert, E. (1987) *Endocrinology* **120,** 707–713.
6. Thompson, R., Seasholtz, A., Douglass, J. & Herbert, E. (1987) *Mol. Endocrinol.* **1,** 363–370.
7. Yoon, D. J., Sklar, C. & David, R. (1988) *Endocrinology* **122,** 759–761.
8. Persson, H., Rehfeld, J. F., Ericsson, A., Schalling, M., Pelto-Huikko, M. & Hökfelt, T. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 6166–6170.
9. Schalling, M., Persson, H., Pelto-Huikko, M., Ödum, L., Ekman, P., Gottlieb, C., Hökfelt, T. & Rehfeld, J. F. (1990) *J. Clin. Invest.* **86,** 660–669.
10. Persson, H., Pelto-Huikko, M., Metsis, M., Söder, O., Brené, S., Skog, S., Hökfelt, T. & Ritzén, E. M. (1990) *Mol. Cell. Biol.* **10,** 4701–4711.
11. Sastry, B. V. & Sadavongvivad, C. (1978) *Pharmacol. Rev.* **30,** 65–132.
12. Bishop, M. R., Sastry, B. V., Schmidt, D. E. & Harbison, R. D. (1976) *Biochem. Pharmacol.* **25,** 1617–1622.
13. Bishop, M. R., Sastry, B. V. & Stavinoha, W. B. (1977) *Biochim. Biophys. Acta* **500,** 440–444.
14. Nelson, L. (1964) *J. Reprod. Fertil.* **7,** 65–71.
15. Nelson, L. (1967) in *Fertilization*, eds. Metz, C. B. & Monroy, A. (Academic, New York), pp. 22–97.
16. Chakraborty, J. & Nelson, L. (1974) *J. Reprod. Fertil.* **38,** 359–367.
17. Stewart, T. A. & Forrester, I. T. (1978) *Biol. Reprod.* **19,** 271–279.
18. Nelson, L. (1976) *Exp. Cell Res.* **101,** 221–224.
19. Placzek, R., Krassnigg, F. & Schill, W. B. (1988) *Arch. Androl.* **21,** 1–10.
20. Dwivedi, C. & Long, N. J. (1989) *Biochem. Med. Metab. Biol.* **42,** 66–70.
21. Harbison, R. D., Dwivedi, C. & Evans, M. A. (1976) *Toxicol. Appl. Pharmacol.* **35,** 481–490.
22. Sastry, B. V., Janson, V. E. & Chaturvedi, A. K. (1981) *J. Pharmacol. Exp. Ther.* **216,** 378–384.
23. Goodman, D. R. & Harbison, R. D. (1981) *Biochem. Pharmacol.* **30,** 1521–1528.
24. Goodman, D. R., Adatsi, F. K. & Harbison, R. D. (1984) *Chem. Biol. Interact.* **49,** 39–53.
25. Casillas, E. R. (1972) *Biochim. Biophys. Acta* **280,** 545–551.
26. Rossier, J. (1977) *Int. Rev. Neurobiol.* **20,** 283–337.
27. Beaumont, H. (1960) *Int. J. Radiat. Biol.* **2,** 247–256.
28. Jackson, A. E., O'Learly, P. C., Ayers, M. M. & de Kretser, D. M. (1986) *Biol. Reprod.* **2,** 425–437.
29. Waynforth, H. B. (1980) *Experimental and Surgical Techniques in the Rat* (Academic, New York), pp. 147–150.
30. Poyet, P. & Labrie, F. (1985) *Mol. Cell. Endocrinol.* **42,** 283–288.
31. Ernfors, P., Hallböök, F., Ebendal, T., Shooter, E., Radeke, M. J., Misko, T. P. & Persson, H. (1988) *Neuron* **1,** 983–996.
32. Brice, A., Berrard, S., Raynaud, B., Ansieau, S., Coppola, T., Weber, M. J. & Mallet, J. (1989) *J. Neurosci. Res.* **23,** 266–273.
33. Ibáñez, C. F., Ernfors, P. & Persson, H. (1990) *J. Neurosci. Res.*, in press.
34. Joseph, D. R., Hall, S. H. & French, F. S. (1985) *J. Androl.* **6,** 392–395.
35. Schalling, M., Dagerlind, Å., Brené, S., Hallman, H., Djurfeldt, M., Persson, H., Terenius, L., Goldstein, M., Schlessinger, D. & Hökfelt, T. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 8306–8310.
36. Amara, S. G., Arriza, J. L., Leff, S. E., Swanson, L. W., Evans, R. M. & Rosenfeld, M. G. (1985) *Science* **229,** 1094–1097.
37. German, D. C., Bruce, G. & Hersh, L. B. (1985) *Neurosci. Lett.* **61,** 1–5.
38. Ishii, K., Oda, Y., Ichikawa, T. & Deguchi, T. (1990) *Mol. Brain Res.* **7,** 151–159.
39. Chen, C. L., Mather, J. P., Morris, P. L. & Bardin, C. W. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 5672–5675.
40. Söderström, K. O. & Parvinen, M. (1976) *Mol. Cell. Endocrinol.* **5,** 181–199.
41. Hecht, N. B. (1989) in *Perspectives of Andrology*, ed. Serio, M. (Raven, New York), pp. 25–36.
42. Söderström, K. O. & Parvinen, M. (1976) *J. Cell Biol.* **70,** 239–246.
43. Rabbitts, P. H., Forster, A., Stinson, M. A. & Rabbitts, T. H. (1985) *EMBO J.* **4,** 3727–3733.
44. Jones, T. R. & Cole, M. D. (1987) *Mol. Cell. Biol.* **7,** 4513–4521.
45. Schubach, W. H. & Horvath, G. (1988) *Nucleic Acids Res.* **16,** 11171–11186.
46. Bellve, A. R. (1979) in *Oxford Reviews of Reproductive Biology*, ed. Finn, C. A. (Clarendon, Oxford), pp. 159–261.
47. Matsumoto, A. (1989) in *The Testis: Comprehensive Endocrinology*, eds. Burger, H. & de Kretser, D. (Raven, New York), pp. 181–196.
48. Ritzén, E. M., Hansson, V. & French, F. S. (1989) in *The Testis: Comprehensive Endocrinology*, eds. Burger, H. & de Kretser, D. (Raven, New York), pp. 262–302.
49. Kasson, B. G. & Hsueh, A. J. (1985) *Endocrinology* **117,** 1874–1880.
50. Nelson, L. (1972) *Exp. Cell Res.* **74,** 269–274.
51. Eusebi, F., Mangia, F. & Alfei, L. (1979) *Nature (London)* **277,** 651–653.
52. Eusebi, F., Pasetto, N. & Siracusa, G. (1984) *J. Physiol. (London)* **346,** 321–330.