Expression and Localization of Gastrin Messenger RNA and Peptide in Spermatogenic Cells

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Abstract

In previous studies we have shown that the gene encoding cholecystokinin (CCK) is expressed in spermatogenic cells of several mammalian species. In the present study we show that a gene homologous to the CCK-related hormone, gastrin, is expressed in the human testis. The mRNA hybridizing to a human gastrin cDNA probe in the human testis was of the same size (0.7 kb) as gastrin mRNA in the human antrum. By in situ hybridization the gastrinlike mRNA was localized to seminiferous tubules. Immunocytochemical staining of human testis revealed gastrinlike peptides in the seminiferous tubules primarily at a position corresponding to spermatids and spermatozoa. In ejaculated spermatozoa gastrinlike immunoreactivity was localized to the acrosome. Acrosomal localization could also be shown in spermatids with electron microscopy. Extracts of the human testis contained significant amounts of progastrin, but no bioactive amidated gastrins. In contrast, ejaculated sperm contained mature carboxyamidated gastrin 34 and gastrin 17. The concentration of gastrin in ejaculated human spermatozoa varied considerably between individuals. We suggest that amidated gastrin (in humans) and CCK (in other mammals) are released during the acrosome reaction and that they may be important for fertilization. (*J. Clin. Invest.* 1990. 86:660–669.) Key words: activation • cholecystokinin • fertilization • neuropeptide • sperm

Introduction

The gastrointestinal hormones gastrin and cholecystokinin (CCK)[1] are homologous. They share an identical carboxyl-terminus Gly-Trp-Met-Asp-Phe-NH$_2$, which contains the active site of both hormones (1, 2). The active site homology explains the overlapping spectra of activity and suggests that gastrin and CCK are derived from a common ancestor (3). This suggestion is supported by analysis of mammalian gastrin and CCK preprohormone structures (4–9) and by the recent detection of an invertebrate hybrid of mammalian gastrin and CCK (10).

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1. *Abbreviations used in this paper:* CCK, cholecystokinin; proCCK, procholecystokinin.

Gastrin and CCK peptides are synthesized throughout the body in different cell types (for review, see Rehfeld [11]). The gastrin gene is expressed mainly in the antroduodenal G cells, and to a minor extent in the neonatal pancreas (12), vagal neurons (13), pituitary cells (14), and bronchial mucosal cells (15). In addition to I cells of the small intestine, the CCK gene is expressed in multiple central (16–19) and peripheral neurons (20), in pituitary corticotrophs (21) and, as recently reported, in spermatogenic cells of several mammalian species (22, 23).

The CCK peptides in spermatogenic cells are accumulated in the acrosome (22–24). CCK receptors have been detected on frog oocytes (25), and we have suggested that accumulated CCK peptides in the acrosome of spermatids and spermatozoa may be released during the acrosome reaction and participate in the fertilization process. Consequently, it became pertinent to examine human spermatogenic cells also for expression of the CCK gene. As shown here, human spermatogenic cells express instead an mRNA hybridizing to a human gastrin cDNA clone of the same size as gastrin mRNA in the antrum. In agreement with this, progastrin was found in the human testis, and bioactive α-amidated gastrin was detected in ejaculated human sperm cells. Thus, human spermatogenic cells appear to express the gene encoding the CCK-related hormone gastrin, rather than CCK expressed in spermatogenic cells of other mammals (22, 23).

Methods

RNA blot analysis. Total RNA was prepared from the indicated tissues by homogenization in 4 M guanidine isothiocyanate, 0.1 M β-mercaptoethanol, 0.025 M sodium citrate (pH 7.0) followed by centrifugation through a cushion of CsCl (26). Polyadenylated RNA was purified by oligo (dT)-cellulose chromatography (27), electrophoresed in 1% agarose gels containing 0.7% formaldehyde, and transferred to nitrocellulose filters, as previously described (26). The filters were then hybridized to a 600-bp Alu 1 fragment including the second exon of the human CCK gene (8) or a 300-bp human gastrin cDNA insert (5) labeled with [α-^{32}P] dCTP by nick-translation to a specific activity of ~ 5 × 10^8 cpm/μg. Filters containing RNA prepared from monkey and human tissues were first washed at reduced stringency (0.2× SSC 0.1% SDS, 48°C; 1× SSC is 150 mM NaCl, 15 mM sodium citrate, pH 7.0) and later, after an initial exposure to x-ray film, at high stringency (0.1× SSC, 0.1% SDS, 54°C). The filters were exposed to XAR-5 films (Eastman Kodak Co., Rochester, NY) after both washes using intensifying screens (DuPont Co., Wilmington, DE).

In situ hybridization. Testes from human and grey monkey were collected with < 1 h of postoperative delay, immersed in 4% paraformaldehyde in phosphate buffer containing 0.3% picric acid (28) for 2–3 h, and transferred to 10% sucrose in 0.1 M phosphate buffer and left overnight. Sections were cut in a cryostat (Dittes, Heidelberg,

FRG) and thawed onto glass slides dipped in poly-L-lysine (100 mg/liter). The sections were then hybridized to three oligonucleotides complementary to, respectively, rat and human CCK, human tyrosine hydroxylase, and human gastrin mRNA, as previously described (29), with the exception that sections were hybridized directly after thawing without any pretreatment or immersion in fixative. The oligonucleotides were synthesized on a DNA synthesizer (model 381A, Applied Biosystems, Inc., Foster City, CA), purified with oligonucleotide purification cartridges (Applied Biosystems, Inc.) and labeled at their 3'-end using terminal deoxynucleotidyl transferase (International Biotec Inc., New Haven, CT) and [α-^{35}S] dATP (New England Nuclear, Boston, MA) to a specific activity of $7-10 \times 10^8$ cpm/μg. The oligonucleotides were complementary to nucleotides 265–308 of human CCK mRNA (9), 151–211 of human gastrin (5), and 1–48 of human tyrosine hydroxylase (30).

After hybridization, sections were washed at 55°C in $1 \times$ SSC for 60 min with four changes, transferred through distilled water and 60% and 95% ethanol, air-dried, and exposed to x-ray film for 4 d (antrum) and 34 d (testis). Subsequently, NTB2 (Kodak) nuclear track emulsion was applied, and finally the sections were stained with cresyl violet. The x-ray film was developed for 2 min in LX 24 (Kodak) and fixed in L4 (Kodak) for 15 min. The nuclear track emulsion was developed in D19 (Kodak) for 2.5 min and fixed in G333 (Agfa Gefvert, Leverkusen, FRG) for 4 min. The autoradiograms were analyzed in a Mikrophot-FX microscope (Nikon Corp., Tokyo) using dark field illumination.

Measurement of progastrin, proCCK, and their products. Human testes were sampled < 1 h postoperatively and frozen in liquid nitrogen. The material was taken from patients undergoing surgery for prostate cancer except one case (27-yr-old male), where surgery was performed because of a testicular tumor. Testis and epididymis from gray monkeys were dissected and immediately frozen on dry ice. The sperm was obtained from voluntary donors and frozen immediately. While frozen, the tissue was immersed in boiling water for 20 min, homogenized, and centrifuged at 10,000 *g* for 30 min. The supernatant (neutral water extract) was stored at −20°C. The pellet was reextracted in 0.5 M acetic acid, rehomogenized, and centrifuged and the supernatant (the acid extract) was stored at −20°C. The extracts were assayed with sequence-specific CCK and gastrin radioimmunoassays before and after incubation with trypsin and carboxypeptidase B as previously described (31). Neutral water extracts were applied, separately or together, to Sephadex G-50 superfine columns (Pharmacia, Uppsala, Sweden) before and after trypsin and carboxypeptidase B treatment. The columns were eluted with 0.02 M barbital buffer (pH 8.4) and calibrated with extracts of jejunal mucosa and cerebral cortex, with sulfated and nonsulfated CCK-8, glycine-extended CCK-8, and CCK-12 (CRB, Cambridge, UK), with carboxy-amidated CCK-33,

gastrin 34 and gastrin 17. Void and total volumes of the columns were determined with ^{125}I-labeled albumin and ^{22}NaCl.

Immunohistochemistry. Tissue treatment was as described above for in situ hybridization except that the tissue sections (14 μm thick) were thawed onto gelatin-chromalum–coated glass slides, after which they were processed for indirect immunofluorescence histochemistry (see Coons [32]). To stain ejaculated spermatozoa a smear preparation was fixed with the "double pH method" of Berod et al. (33) as modified by Pelto-Huikko et al. (24). The smear preparations were first fixed with 4% paraformaldehyde at pH 6.5 for 5 min followed by the same fixative at pH 11 for 15 min. A rabbit polyclonal antiserum directed towards the common carboxyl-terminal sequence of CCK-8 and gastrin (18, 34) and mouse monoclonal antibodies directed against the common carboxyl-terminal sequence of CCK and gastrin (J. Walsh, unpublished method; see reference 19) were used, followed by fluorescein isothiocyanate (FITC)-conjugated goat anti–rabbit antibodies (Boehringer Mannheim Scandinavia, Stockholm, Sweden) or FITC-conjugated sheep anti–mouse antibodies (Amersham, Amersham, UK). As a control the sections were incubated with antiserum preadsorbed with 1 μM synthetic CCK-8 peptide (Peninsula Laboratories, Inc., Belmont, CA) for 24 h at 4°C. After mounting in phosphate-buffered glycerol containing para-phenylenediamine (35, 36), the slides were examined in a Mikrophot-FX microscope equipped for epifluorescence and with appropriate filter combinations.

Immunoelectron microscopy. Human testes were sliced and immersion fixed in a mixture of paraformaldehyde (4%) and glutaraldehyde (0.2%) in 0.1 M phosphate buffer (pH 7.3) for 6 h. The tissues were cryoprotected with 30% sucrose over night, and 50-μm frozen sections were cut. The sections were stained free floating using the pre-embedding modification (37) of the peroxidase-antiperoxidase method (38). Endogenous peroxidase activity was blocked by incubating the sections in 0.5% H_2O_2 in methanol for 30 min. To increase the antibody penetration 0.2% Triton X-100 was applied for 5 min. Nonspecific binding of the antisera was blocked by pretreating the sections for 30 min with 10% normal rabbit serum. The sections were incubated for 10–16 h with the mouse monoclonal CCK antibodies (see above) diluted 1:2,000. After incubation with appropriate secondary antibodies peroxidase activity was visualized with a solution of 0.03% diaminobenzidine and 0.004% H_2O_2 in PBS. The sections were postfixed with 2% aqueous OsO_4 for 60 min and *en bloc* stained with 1% uranyl acetate for 60 min. Sections were dehydrated through graded ethanol and embedded in agar 100 resin. Semithin sections (10 μm thick) were cut from blocks and attached to glass slides by warming. The sections were inspected in a light microscope, and a capsule filled with agar 100 was inverted on the sections showing immunostaining. After polymerization the capsule was detached. Ultrathin sections were cut on an OMU-3 ultramicrotome (Reichert, Vienna, Austria) and collected on
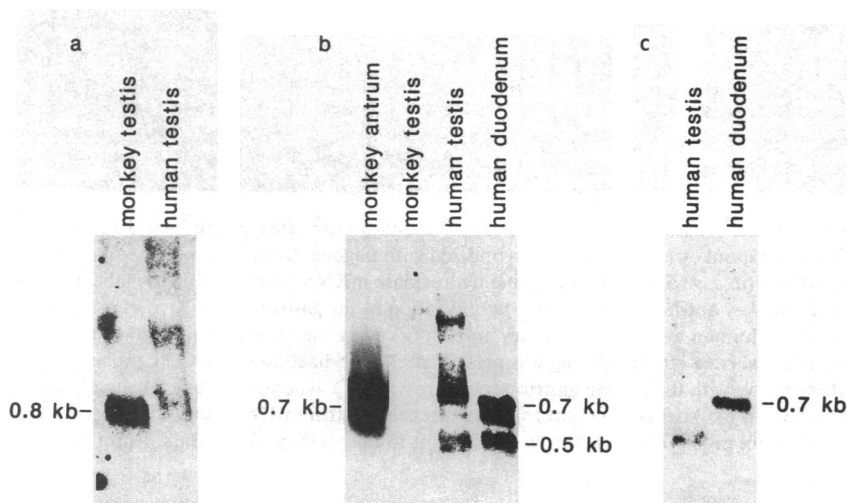

Figure 1. Expression of gastrinlike mRNA in monkey and human testis. Polyadenylated RNA was prepared from the indicated tissues, and 20 μg from each sample was electrophoresed in a formaldehyde-containing agarose gel blotted onto nitrocellulose and hybridized to a human CCK probe (*a*) and a human gastrin probe (*b* and *c*). The filters in *a* and *b* were washed at reduced stringency (0.2× SSC, 0.1% SDS, 48°C), whereas the filter shown in *c* was washed at high stringency (0.1× SSC, 0.1% SDS 54°C). After washing the filters were exposed to x-ray film using a DuPont intensifying screen.
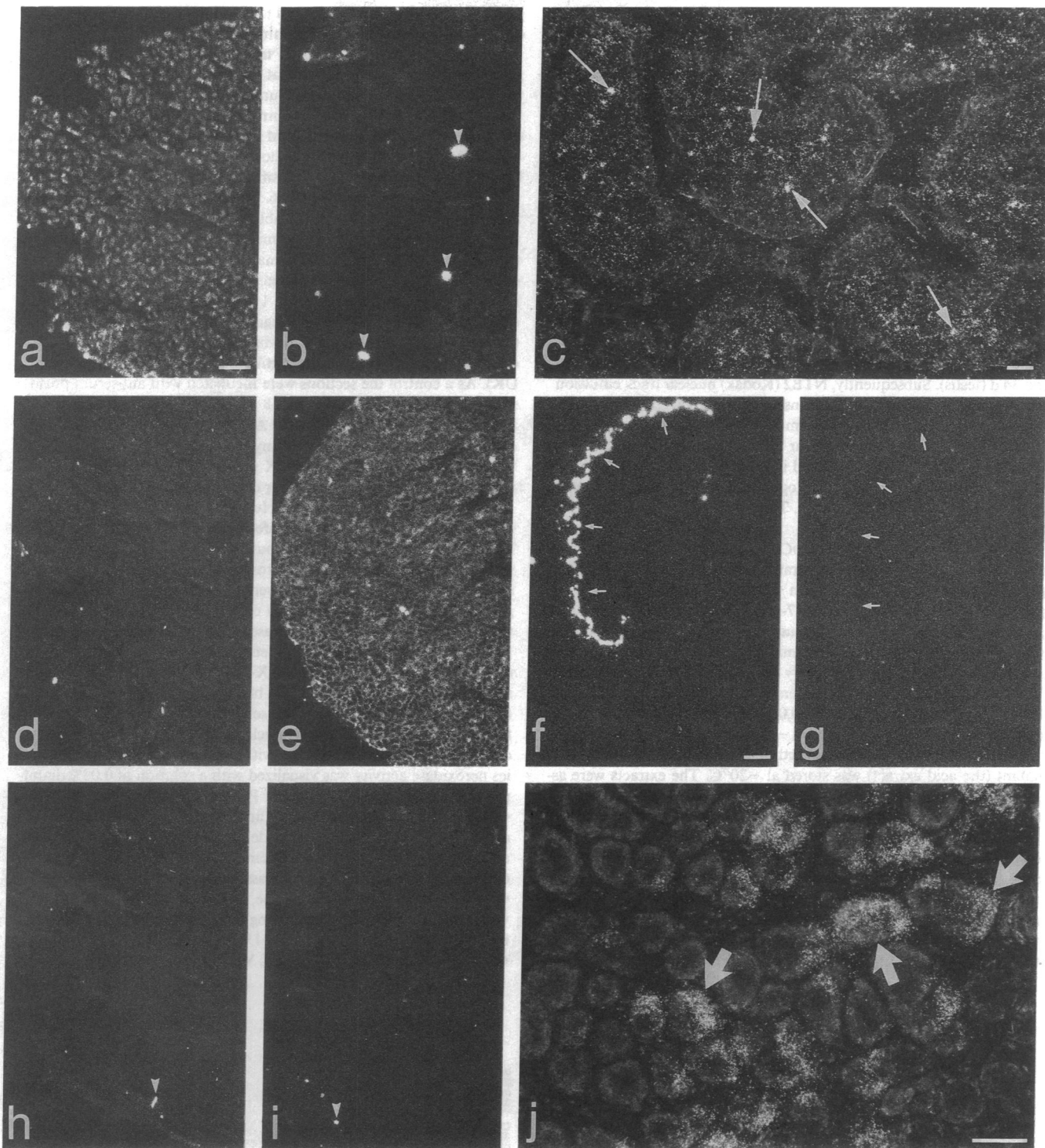
Figure 2. (*a, b, d–i*) Film and (*c* and *j*) emulsion autoradiographs of sections through human and monkey testes after in situ hybridization. Sections of human (*a, c, d, h*) and grey monkey (*b, e, i*) testis and monkey antrum (*f, g, j*) hybridized with oligonucleotides complementary to human gastrin mRNA (*a–c, f, j*), rat and human CCK mRNA (*d, e, g*) and human tyrosine hydroxylase mRNA (*h* and *i*). Intense labeling was seen overlying human seminiferous tubules (*a* and *c*) and monkey antrum (*f* and *j*) after hybridization to the gastrin probe. Arrows in *c* point to structures with high activity. No labeling can be seen in the human testis (*d*) or monkey antrum (*g*) using the CCK probe. Monkey testis hybridized with the gastrin probe shows little or no labeling (*b*), whereas strong labeling was present after hybridization to the CCK probe (*e*). Note band of labeled cells in monkey antrum after hybridization with the human gastrin probe (arrows in *f*), whereas no such labeling is seen in the corresponding place with a human CCK probe (arrows in *g*). Arrows in *j* point to cells expressing gastrin mRNA (emulsion-dipped section). No labeling is seen using tyrosine hydroxylase as a control probe (*h* and *i*). Arrow heads point to unspecific activity. Bars, 1 mm (*a, b, d–i*) or 50 μm (*c* and *j*).

Tissue	Progastrins	Glycine-extended gastrins	Carboxyamidated gastrins
		pmol/g tissue, wet wt	
Testicular tissue ($n = 5$)*	5.0 (4.2–5.2)	1.0 (0.8–1.2)	0
Ejaculated spermatozoes ($n = 6$)*	1.1 (0–5.2)	0 (0–2.9)	0.8 (0–3.9)

* Number of samples (from individual males).

100-mesh copper grids, contrasted with uranyl acetate and lead citrate and examined in an electron microscope (model 1200 EX, JEOL, Tokyo, Japan).

Results

Detection of a gastrinlike mRNA in the human testis. Polyadenylated RNA was prepared from human testis and duodenum, as well as from monkey antrum and testis. In agreement with a previous study (23), high levels of an 800-nucleotide-long CCK mRNA were found in monkey testis (Fig. 1 *a*). In contrast, the CCK probe only hybridized weakly to a 0.8-kb transcript in the human testis.

The human gastrin cDNA probe hybridized strongly to a 700-nucleotide-long gastrin mRNA in monkey antrum, but showed no hybridization to any RNA transcript in the monkey testis (Fig. 1 *b*). In the human testis, the gastrin cDNA probe hybridized to a 0.7-kb transcript under reduced stringency (0.2× SSC, 48°C) as well as to a smaller, ~ 0.5-kb-long transcript (Fig. 1 *b*). The same-size transcripts were also seen in the human duodenum using the gastrin cDNA probe. However, after high-stringency washing of the filter (0.1× SSC, 54°C), the levels of the transcripts in the human testis that hybridized to the gastrin cDNA probe were greatly reduced, whereas the 0.7-kb transcript in the human duodenum was still present at a level comparable to that seen under reduced stringency (Fig. 1 *c*). The same results were obtained using RNA preparations from five different individuals ranging in age from 27 to 68 yr old.

In situ hybridization of monkey and human testis. The human testis showed strong labeling over the seminiferous tubules after hybridization to a 61-mer oligonucleotide complementary to human gastrin mRNA (Fig. 2 *a* and *c*), but only background labeling with a 44-mer oligonucleotide probe specific for human CCK mRNA (Fig. 2 *b*). The gastrin probe labeled G-cells of monkey antrum strongly (Fig. 2, *f* and *j*), but this was not seen with the CCK probe (Fig. 2 *g*). In the monkey testis, dense labeling with the CCK probe was found overlying the basal part of the seminiferous tubules, where spermatogonia, spermatides, and Sertoli cells are found (Fig. 2 *e*). In contrast, the gastrin probe did not show any labeling in the monkey testis (Fig. 2 *d*). A control probe, complementary to human tyrosine hydroxylase, with similar specific activity, length, and GC content, which hybridizes strongly to human adrenal medulla tissue (data not shown), did not label either human or monkey testis (Fig. 2, *h* and *i*).

Identification of progastrin, proCCK, and their products in human testes. Using radioimmunoassays specific for different sequences of progastrin and proCCK, progastrin peptides were

detected in the human testis, but no α-carboxyamidated gastrin peptides were measured (Table I). However, extracts of ejaculated human spermatozoa contained α-carboxyamidated gastrin-34 and gastrin-17 peptides in addition to progastrin peptides. ProCCK was measured in extracts of monkey testis and epididymis. Only small amounts of bioactive, mature peptides were found in the extracts. The concentrations of fully processed α-carboxyamidated CCK-8 in monkey testis and epididymis were ~ 100 times lower compared with the duodenal mucosa (Table II). Characterization of the various CCK and gastrin peptides present in monkey and human testes confirmed that the vast majority of these peptides were present in their precursor form (Fig. 3). However, a similar analysis in ejaculated human spermatozoa revealed that the gastrin peptides in these samples corresponded to the bioactive α-carboxyamidated gastrin 34 and gastrin 17 (Fig. 4).

Immunohistochemical localization of gastrin and CCK peptides. An antiserum directed to the carboxyl-terminal part common to CCK and gastrin was used in the present study. Thus, our antiserum cannot distinguish between these two peptides. However, since both hybridization and biochemical studies indicate presence of a gastrinlike peptide, we will throughout this paper use the term gastrinlike immunoreactivity.

Sections from adult human testis revealed small gastrin-immunoreactive structures in the seminiferous tubules predominantly at a position corresponding to the localization of spermatids and spermatozoa (Fig. 5, *a* and *c*). The fluorescent structures were often crest-shaped and sometimes rounded. Different seminiferous tubule profiles showed varying numbers of positive structures with different intensities, and some tubules profiles seemed to lack staining. Both Leydig cells and cells in the peripheral parts of the seminiferous tubules were nonimmunoreactive. In a smear of ejaculated spermatozoa, intensely stained structures were present at the anterior pole of the head of the spermatozoa (cf. Fig. 5, *b* and *d*). The immunoreactive structures had a semicircular and sometimes crest-shaped appearance and were present in a large proportion of spermatozoa, although unlabeled and more weakly labeled structures could be clearly distinguished (Fig. 5, *d* and *f*). Comparing the same smears using phase-contrast microscopy (Fig. 5 *f*), further defined the exact localization of the immunoreactivity within spermatozoa (Fig. 5 *d*). Preadsorption of the antibodies with synthetic CCK-8 peptide abolished the immunoreactivity seen in the human testis (Fig. 5 *e*).

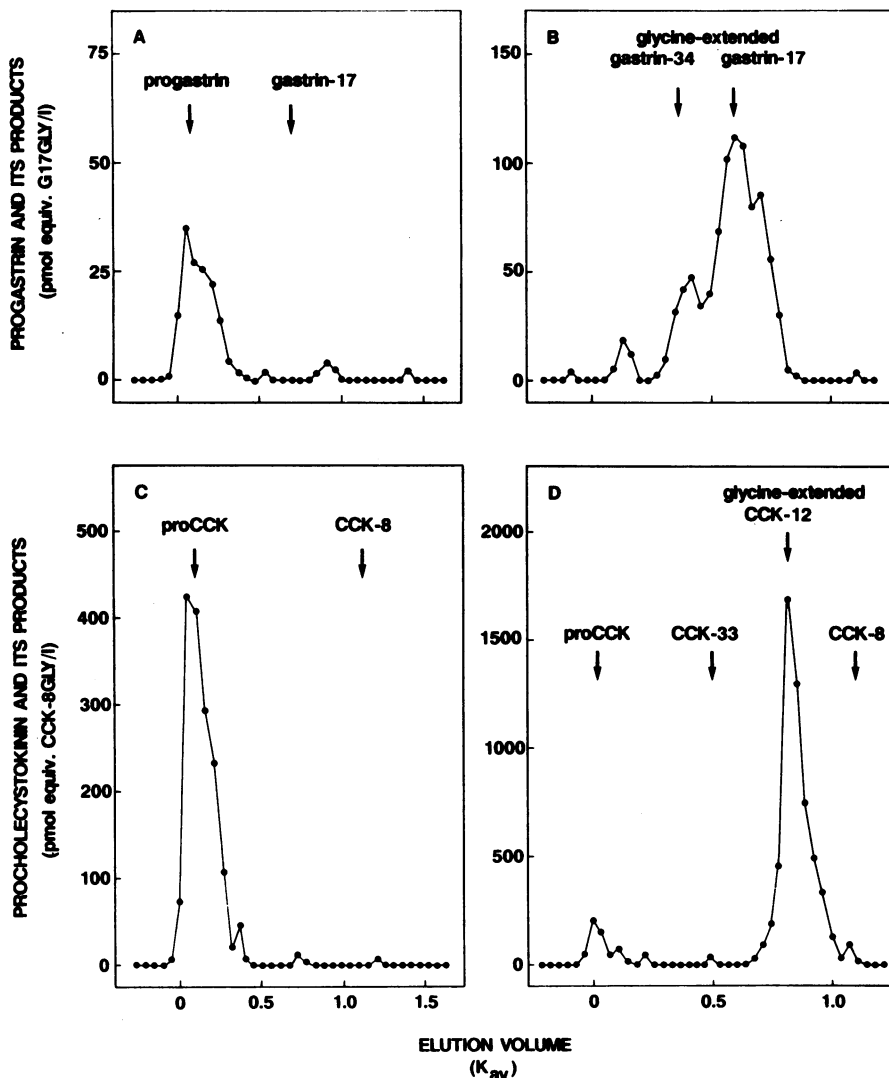| Tissue	Procholecystokinins	Glycine-extended cholecystokinins	Carboxyamidated cholecystokinins
		pmol/g tissue, wet wt	
Testicular tissue ($n = 2$)*	22 (21–23)	1.2 (1.1–1.3)	0.4 (0.3–0.4)
Epididymis ($n = 2$)*	7.9 (6.5–9.3)	0.7 (0.4–1.0)	0.7 (0.7–0.7)

* Number of samples.

Figure 3. Sephadex G50 gel chromatography of extracts from testicular tissue. (*A*) Elution profile of extract of undigested progastrin from human testes. (*B*) Elution profile of extract from human testes after trypsin-carboxypeptidase B digestion of the progastrin before column application. (*C*) Elution profile of extract of undigested procholecystokinin (proCCK) from monkey testis. (*D*) Elution profile of extract from monkey testes after trypsin-carboxypeptidase B digestion of the proCCK before column application. For details of the analytical procedure, see Methods.
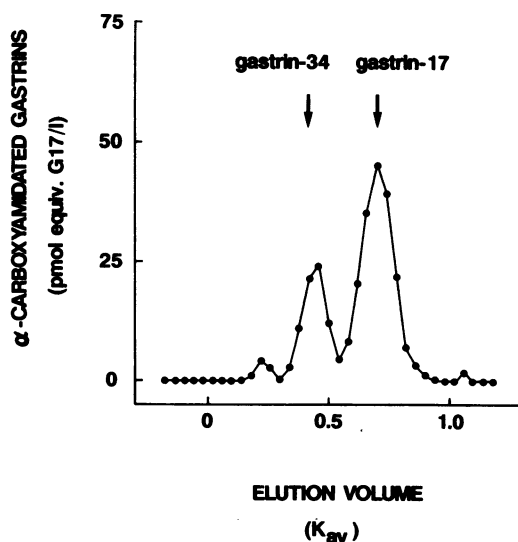

Figure 4. Sephadex G50 gel chromatography of extract of ejaculated human spermatozoa. The elution profile represents undigested gastrin. For details of the analytical and chromatographic procedures, see Methods.

Immunoelectron microscopic localization of the gastrinlike peptides in human spermatogenic cells. At the electron microscopic level the gastrinlike immunoreactivity in the human testis was found in the acrosomal cap of spermatids (Fig. 6, *a* and *b*). No clear staining was observed in the cytoplasm of spermatids. Both Sertoli cells and interstitial Leydig cells were nonimmunoreactive. After preadsorption of the antiserum with synthetic CCK-8 peptide, no labeling of acrosomal vesicles could be seen.

Discussion

The present study shows that progastrin peptides are present in the human testis and that α-carboxyamidated gastrin-17 and gastrin-34 peptides are present in ejaculated human sperms. In agreement with this, a 0.7-kb mRNA species with the same size as gastrin mRNA in the antrum and duodenum was detected in human testis by Northern blot analysis using a human gastrin cDNA probe. Furthermore, a 61-mer oligonucleotide specific for human gastrin mRNA showed labeling over the seminiferous tubules in the human testis using in situ hybridization. In the monkey testis, previously shown to ex-
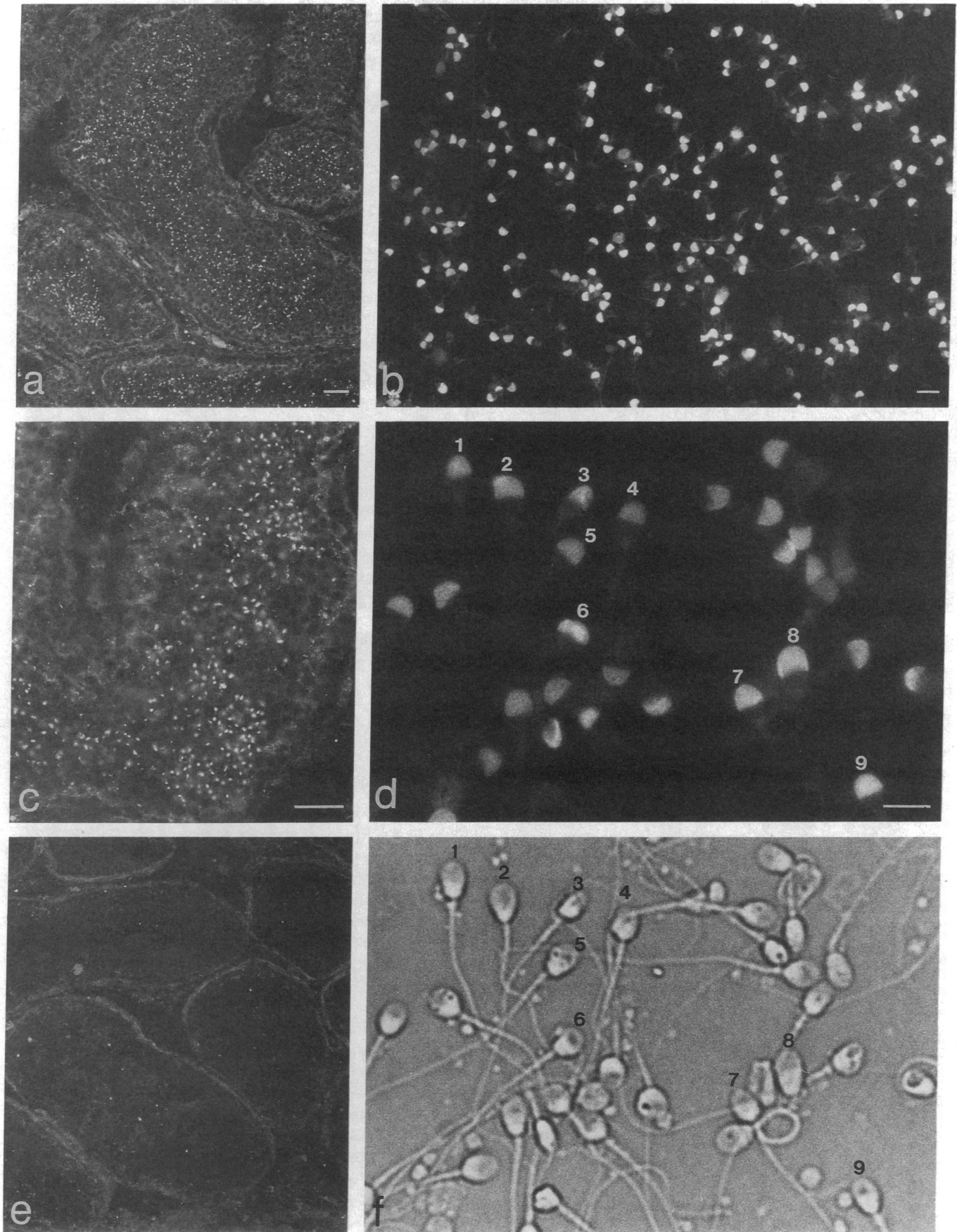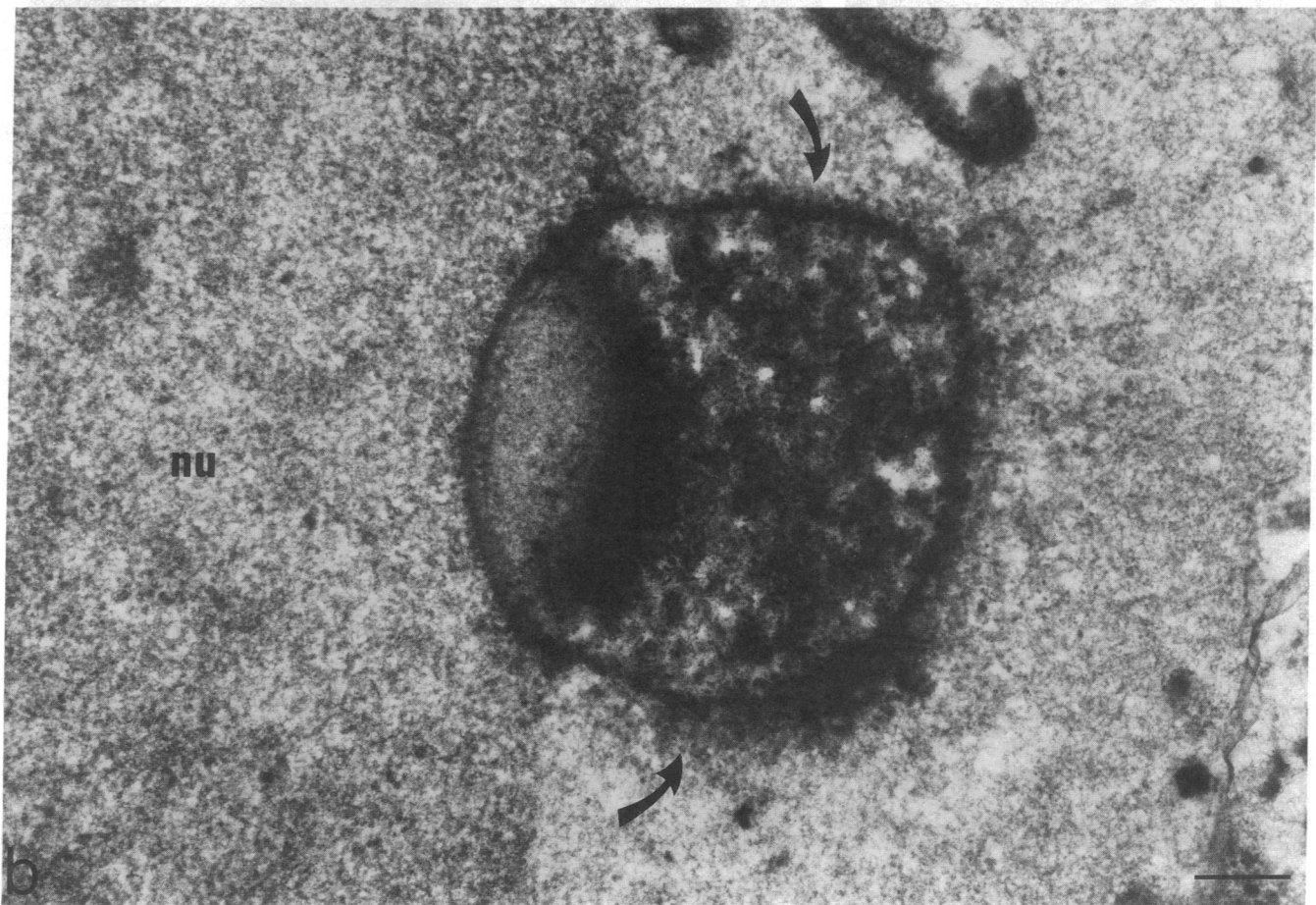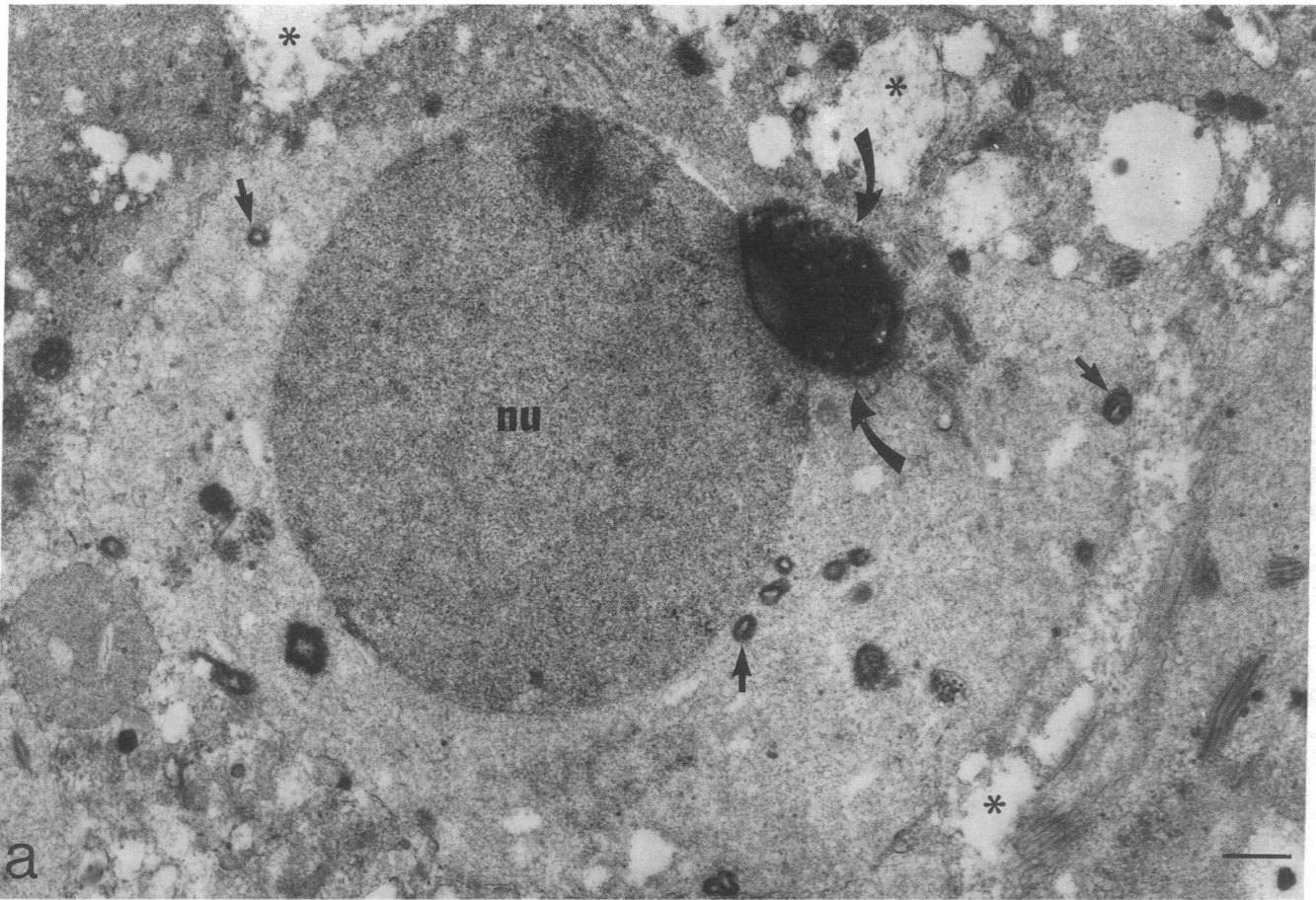
Figure 5.

Figure 6.

Figure 5. Immunohistochemical staining of human testis and ejaculated sperm. (*a–e*) Immunofluorescence and (*f*) phase-contrast micrographs of human testis (*a, c, e*) and smear of human sperm (*b, d, f*) after incubation with antiserum to the carboxyl-terminal portion of gastrin/CCK (*a–d*) or CCK antiserum preabsorbed with CCK octapeptide (*e*). (*f*) Same section as in *d* but viewed in the phase-contrast microscope. Numerous dotlike structures can be seen mainly located in the central portion of the seminiferous tubules (*a* and *c*). After incubation with preabsorbed antiserum, no such staining can be observed (*e*). In a smear of ejaculated sperms, gastrinlike immunoreactivity can be seen in most sperm heads, distinctly located to the anterior pole of the head (*b* and *d*). Comparison with the phase-contrast image (*f*) provides information on the exact localization of gastrinlike immunoreactivity. Some of the sperm cells have been numbered 1–9 for comparison. Bars, 50 μm (*a, c, e*) and 5 μm (*b, d, f*).

Figure 6. Electron micrograph of human spermatid. Electron micrographs of human spermatid (*a* and *b*) after incubation with gastrin/CCK carboxyl-terminal antiserum and processing for the peroxidase antiperoxidase technique. (*a*) Immunoreactivity is localized in the acrosome (*curved arrows*). Mitochondria and multivesicular bodies (*small arrows*) in the cytoplasm exhibit nonspecific osmiofilia. Some fixation processing artefacts are seen (*stars*). (*nu*) Nucleus. Bar, 1 μm. (*b*) Higher magnification of a labeled acrosome. Immunoreaction is seen in the acrosomal vesicle and in the acrosomal granule. (*nu*) Nucleus. Bar, 0.2 μm.

press CCK mRNA (23), the human gastrin cDNA probe did not hybridize to any RNA transcript, whereas the same probe revealed high levels of a 0.7-kb gastrin mRNA in the monkey antrum. Similarly, in situ hybridization experiments revealed no labeling over sections from monkey testis using the human gastrin-specific oligonucleotide, whereas an oligonucleotide specific for human CCK mRNA showed labeling over the peripheral parts of the seminiferous tubules in this species. Hence, it appears that the gastrin gene is expressed in human spermatogenic cells, whereas in monkey, as well as in rat, mouse, guinea pig and pig spermatogenic cells, the gene encoding the gastrin related peptide CCK is expressed (23).

To facilitate the possible detection of gastrin mRNA in the testis of other species than the human (monkey, mouse, rat, guinea pig, and pig) all RNA blots were initially examined after washing at reduced stringency (0.2× SSC, 48°C). At this stringency a rat gastrin cDNA clone with 73% nucleotide sequence homology to human gastrin cDNA hybridizes to gastrin mRNA from human antrum (9). In spite of this, the 0.7-kb mRNA that hybridized to the human gastrin cDNA probe was only detected in human testis, suggesting that the gastrin gene is not expressed in the testis of all other species analyzed. Surprisingly, however, the 0.7-kb gastrin mRNA in human testis was barely detected, when the filters had been washed at high stringency (0.1× SSC, 54°C). This suggests, in spite of the finding of gastrin peptides in human testis and sperms, that the 0.7-kb RNA transcript seen in the human testis does not correspond to authentic gastrin mRNA. Instead this transcript may represent a novel mRNA with relatively high sequence homology to human gastrin mRNA. This interesting possibility should be clarified by the isolation and characterization of a cDNA clone encoding the gastrinlike mRNA detected in human testis.

As observed in the rat testis and epididymis (23), the majority of the CCK peptides in the monkey testis and epididymis represented proCCK peptides. In the epididymis, processed glycine-extended and carboxyamidated CCK peptides represented a larger proportion of the detected CCK peptides than in the testis (23). Also, all gastrin peptides detected in the human testis were in the precursor form. However, in ejaculated human sperm cells, the majority of the gastrin peptides represented fully processed gastrin-17 and gastrin-34 peptides corresponding to the bioactive forms seen in the antral mucosa. These data suggest that the CCK and gastrin peptides expressed in spermatogenic cells to some extent are processed to their biologically active forms within these cells, and that the

majority of this processing occurs when the spermatozoa matures in the epididymis. Thus, biologically active, fully processed CCK and gastrin peptides appear to be important in the distal parts of the genital tract. The possibility that the proCCK and progastrin peptides have a biological function locally in the testis can, however, not be excluded.

The antiserum used in this study for immunohistochemical staining of sections from human testis recognizes the pentapeptide Gly-Trp-Met-Asp-Phe-NH₂ common to both CCK and gastrin. The observed immunoreactivity can, therefore, represent either CCK or gastrin or both. Reactivity against a yet unidentified peptide containing this pentapeptide sequence can also not be excluded. However, based on the biochemical characterization of gastrin peptides in extracts of human testis, it appears most likely that the immunoreactivity seen represents progastrin peptides or possibly also processed α-carboxyamidated gastrin 17 or gastrin 34. As seen for the CCK-like immunoreactivity in mouse, rat, and monkey testis (22–24), the gastrinlike immunoreactivity in the human testis was localized to the central parts of the seminiferous tubules, where spermatids and spermatozoa are present. In addition, ejaculated spermatozoa were stained in the anterior part, suggesting an acrosomal localization. The results of the subcellular localization by immunoelectron microscopy showed gastrinlike immunoreactivity in the acrosome of human spermatids. A similar analysis of monkey spermatids demonstrated CCK-like immunoreactivity in the acrosome (23). The fact that both immunoreactivities were localized to the acrosome in monkeys and in humans, respectively, suggests that the two peptides have similar function(s) in the male reproductive system. This is further supported by the fact that the biologically active site in CCK and gastrin resides in their carboxyl-terminal region, i.e., the region which is structurally identical for both peptides.

The acrosome contains enzymes of lysosomal nature which are released during the acrosome reaction, thereby facilitating sperm activation of the egg (39). As discussed earlier (22, 23), an interesting possibility is that CCK, or gastrin peptides in humans, are released during the acrosome reaction to interact with receptors on the follicular cells or on the egg cell membrane, thereby activating the egg for fertilization. In support of this hypothesis, Moriarty et al. (25) recently demonstrated the presence of functional CCK receptors on *Xenophus Laevis* oocyte cell membranes. Furthermore, Kline et al. (40) recently suggested that there may be an endogenous receptor in the egg membrane that is activated by sperm to initiate

fertilization, and they showed that the serotonin or M1 muscarinic acetylcholine receptors could mimic the putative endogenous receptor. Our finding of CCK or gastrin peptides in the acrosome of spermatids or spermatozoa suggests that these peptides may be components that activate the egg for fertilization, provided that functional receptors can be demonstrated also on mammalian oocytes. Validation of this hypothesis may come from experiments studying the effect of CCK and gastrin agonists or antagonists on fertilization. In addition, the presence of gastrin peptides in human spermatozoa opens possibilities to study their effects on in vitro fertilization in humans.

Acknowledgments

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