Journal of Neurochemistry
Raven Press, Ltd., New York

Glutamate Decarboxylases in Nonneural Cells of Rat Testis and Oviduct: Differential Expression of GAD_{65} and GAD_{67}

*Niranjala J. K. Tillakaratne, †Mark G. Erlander, ‡Michael W. Collard, §Karen F. Greif, and *‖¶Allan J. Tobin

**Department of Biology, †Neuroscience Program, ‖Molecular Biology Institute, and ¶Brain Research Institute, University of California, Los Angeles, Los Angeles, California; ‡Mental Health Research Institute, University of Michigan, Ann Arbor, Michigan; and §Department of Biology, Bryn Mawr College, Bryn Mawr, Pennsylvania, U.S.A.*

Abstract: γ-Aminobutyric acid (GABA) and its synthetic enzyme, glutamate decarboxylase (GAD), are not limited to the nervous system but are also found in nonneural tissues. The mammalian brain contains at least two forms of GAD (GAD_{67} and GAD_{65}), which differ from each other in size, sequence, immunoreactivity, and their interaction with the cofactor pyridoxal 5'-phosphate (PLP). We used cDNAs and antibodies specific to GAD_{65} and GAD_{67} to study the molecular identity of GADs in peripheral tissues. We detected GAD and GAD mRNAs in rat oviduct and testis. In oviduct, the size of GAD, its response to PLP, its immunoreactivity, and its hybridization to specific RNA and DNA probes all indicate the specific expression of the GAD_{65} gene. In contrast, rat testis expresses the GAD_{67} gene. The GAD in these two reproductive tissues is not in neurons but in nonneural cells. The localization of brain GAD and GAD mRNAs in the mucosal epithelial cells of the oviduct and in spermatocytes and spermatids of the testis shows that GAD is not limited to neurons and that GABA may have functions other than neurotransmission. **Key Words:** Glutamate decarboxylase—γ-Aminobutyric acid—Oviduct—Testis—mRNAs. **Tillakaratne N. J. K. et al.** Glutamate decarboxylases in nonneural cells of rat testis and oviduct: Differential expression of GAD_{65} and GAD_{67}. *J. Neurochem.* **58,** 618–627 (1992).

γ-Aminobutyric acid (GABA) is a multifaceted molecule. It is the major inhibitory neurotransmitter in the vertebrate brain and may also serve as a neurotransmitter in the vertebrate PNS (Jessen et al., 1979). GABA is also involved as a neurotrophic factor during neural development (Redburn and Schousboe, 1987). GABA is not limited to the nervous system, however, but is present in many nonneural tissues, where it may serve diverse physiological functions (for reviews, see Erdö and Kiss, 1986; Erdö and Wolff, 1990). For example, in the pancreas, GABA produced in β cells inhibits glucagon secretion by α cells (Rorsman et al., 1989), and in the testes GABA stimulates production of testosterone (Ritta et al., 1987). GABA thus is an important signaling molecule in both neural and nonneural systems.

The synthesis of GABA in brain depend mainly on the activity of glutamate decarboxylase (GAD; L-glutamic 1-carboxylase; E.C. 4.1.1.15). Several groups have demonstrated GAD heterogeneity with respect to molecular size, interaction with pyridoxal phosphate (PLP), and subcellular localization (Denner and Wu, 1985; Spink et al., 1985; Legay et al., 1987; Chang and Gottlieb, 1988; reviewed by Martin, 1987; Erlander and Tobin, 1991). This laboratory has recently established that rat brain contains two forms of GAD, with molecular sizes of 65,000 and 67,000. These GADs (GAD_{65} and GAD_{67}, respectively) are the products of two genes, each of which is present in a single copy in the rat genome (Erlander et al., 1991).

The molecular identity of nonneural GAD, however, remains obscure. Reports from the last 22 years indicate that properties of nonneural GADs resemble brain GADs to differing extents, depending on the tissue examined. For example, immunological and enzymatic studies suggest that GADs in oviduct, adrenal chromaffin cells, and pancreatic β cells are similar to brain GAD, whereas GAD species in heart, kidney, and liver are different (Wu and Roberts, 1973; Wu, 1977; Wu et al., 1978, 1986*b*; Vincent et al., 1983; Apud et al.,

Received March 13, 1991; revised manuscript received June 26, 1991; accepted June 28, 1991.

Address correspondence and reprint requests to Dr. A. J. Tobin at Department of Biology, University of California, Los Angeles, Los Angeles, CA 90024-1606, U.S.A.

Abbreviations used: GABA, γ-aminobutyric acid; GAD, glutamate decarboxylase; GAD_{65} and GAD_{67}, glutamate decarboxylase having molecular sizes of 65,000 and 67,000, respectively; PLP, pyridoxal phosphate; SDS, sodium dodecyl sulfate; SSC, saline-sodium citrate.

1984; Erdö et al., 1984, 1989; Kataoka et al., 1984; Baekkeskov et al., 1990). An antiserum to rat brain GAD (Oertel et al., 1981*b*) shows immunoreactivity with the mucosal epithelial cells in the rat oviduct (Erdö et al., 1989). GAD activity in renal cortex does not depend on PLP to the same extent as in brain, adrenal chromaffin cells, and oviduct (Whelan et al., 1969; Oertel et al., 1981*a*; Goodyer et al., 1982; Kataoka et al., 1984; Erdö et al., 1984).

Isolation and characterization of a brain GAD cDNA (Kaufman et al., 1986) allowed us to study nonneural GAD using molecular hybridization techniques. Using this cDNA (which encodes GAD_{67}), we have demonstrated the presence of GAD_{67} RNAs in the testes of rats (Tillakaratne and Tobin, 1986; Tobin, 1989). GAD_{67} RNAs are also present in mice, guinea pigs, monkeys, and humans and are specifically localized in the germ cells of the rat testis (Persson et al., 1990). Furthermore, GAD-like immunoreactivity is present in spermatozoa.

Our laboratory has recently found a second form of GAD (GAD_{65}) in brain (Erlander et al., 1991). In view of this finding, we asked whether these two forms of GAD are also present in other tissues. To address this question, we used cDNAs and antibodies specific to either GAD_{65} or GAD_{67} to study the size, distribution, and localization of GAD in nonneural tissues.

MATERIALS AND METHODS

Isolation of rat tissues

Adult and prepubertal Wistar rats were killed after halothane anesthesia, and the tissues were quickly frozen in liquid nitrogen and stored at −70°C. Female animals were randomly cycling.

Cell isolation and culture

Sertoli cell cultures were prepared from 22-day-old rats by the method of Dorrington and Fritz (1975) and cultured at 32°C in Ham's F-12 medium supplemented with 9 m*M* HEPES (pH 7.45), 215 μg/ml of L-glutamine, 50 U/ml of penicillin, 50 μg/ml of streptomycin, 50 μg/ml of gentamicin, and amphotericin B 0.625 μg/ml of (Fungizone). Peritubular (myoid) cultures were prepared from adult testis as described before (Morales et al., 1987) and cultured in Dulbecco's modified Eagle's medium supplemented with 3% horse serum at 37°C. Germinal cells were isolated using sequential collagenase and trypsin treatment, resuspended in F-12 medium supplemented with 6 m*M* pyruvate and 3 m*M* lactate, and fractionated on 1–4% bovine serum albumin gradients in F-12 medium at unit gravity in a Staput apparatus (Miestrish et al., 1973). Isolated cells were routinely monitored by phase-contrast microscopy and usually contained <2% contamination by Sertoli, Leydig, or peritubular cell types.

TM-4, a Sertoli cell line, and LC-540, a Leydig cell line derived from mouse testes, were purchased from the American Type Culture Collection and grown according to their culture conditions.

RNA isolation

Tissues were homogenized in 4 *M* guanidinium thiocyanate, and total RNA was isolated by the method of Chirgwin et al. (1979). Poly(A)$^+$ RNA was isolated by affinity chromatography by two rounds of binding to an oligo(dT) cellulose (Collaborative Research or BRL) as previously described (Wood et al., 1986).

Northern blot hybridization

RNA was treated with formaldehyde, separated by gel electrophoresis in 1% agarose, and transferred to Biotrans nylon membranes (Maniatis et al., 1982). When DNA probes were used, membranes were prehybridized for 4–12 h at 42°C in 50% formamide, 5× saline–sodium citrate (SSC; 1× SSC comprises 0.15 *M* NaCl and 0.015 M sodium citrate, pH 7.0), 50 m*M* sodium phosphate (pH 6.5), 5× Denhardt's solution, 0.5% sodium dodecyl sulfate (SDS), and 250 μg/ml of heat-denatured salmon sperm DNA (Segovia et al., 1990). Hybridizations proceeded for 18–24 h at 42°C in the same solution. After hybridization, blots were washed at room temperature three times for 5 min each in 2× SSC and 0.1% SDS, followed by two 30-min washes at 50°C in 0.1× SSC and 0.1% SDS. Filters were then exposed to Kodak XAR-5 film for 1–3 days at −70°C.

When RNA probes were used, membranes were prehybridized for 2–6 h at 60°C in 400 m*M* sodium phosphate (pH 7.2), 1 m*M* EDTA, 1 mg/ml of bovine serum albumin (fraction V; Sigma), and 50% formamide (Church and Gilbert, 1984; Khrestchatisky et al., 1991). The ^{32}P-labeled RNA probe was added to the prehybridization solution, and, after hybridization at 60°C overnight, the membranes were washed twice for 30 min at room temperature in 2× SSC and 0.1% SDS and twice for 2 h in 0.1× SSC and 0.1% SDS at 72°C. ^{32}P-labeled cDNA probes or RNA probes were added to the hybridization solution at a concentration of $>1–3 \times 10^6$ cpm/ml. After exposure to film, the probe was removed from the blots by washing at 65°C in 50% formamide and 10 m*M* sodium phosphate (pH 6.5) for 1 h. The stripped blots were rinsed in 2× SSC and 0.1% SDS and exposed to film to check for complete removal of probe.

Preparation of cDNA probes

cDNAs were labeled by random priming (Feinberg and Vogelstein, 1984). The GAD_{67} cDNA probe used in this study was isolated from a λgt-11 library of a rat whole brain using the feline GAD_{67} cDNA (Kaufman et al., 1986; Kobayashi et al., 1987). This clone contained all of the coding region, 0.1 kb of the 5′ untranslated region, and 1.0 kb of the 3′ untranslated region. Rat GAD_{65} cDNA contains all the coding region, 0.07 kb of the 5′ untranslated region, and 0.4 kb of the 3′ untranslated region (Erlander et al., 1991).

In situ hybridization

Testes and oviduct were immediately removed from animals killed with halothane and placed in O.C.T. (Tissue-Tek) in embedding molds. Blocks were frozen on dry ice and either sectioned immediately or stored at −70°C. Transfer sections (10 μm) were cut using a cryostat and thaw-mounted onto gelatin-coated slides as previously described (Wuenschell et al., 1986). After air-drying, slides were postfixed for 30 min in 4% paraformaldehyde in phosphate-buffered saline, rinsed, dehydrated, and stored at −70°C with desiccant until processed for in situ hybridization. ^{35}S-labeled cRNA transcripts were prepared from rat GAD_{65} and GAD_{67} cDNAs according to the technique of Wuenschell et al. (1986). Both sense and antisense probes were prepared and subjected to limited alkaline hydrolysis to give probes of 100–200 bases. Probe (3–5 ng) was added in 50 μl of solution. Each slide was then coverslipped and incubated for 16 h at 50°C.

Measurement of GAD activity

GAD activity was measured according to the procedure of Krieger and Heller (1984). The production of $^{14}CO_2$ from decarboxylation of L-[1-^{14}C]glutamate (52.6 Ci/mmol; NEN) was determined in tissue homogenates in the presence or absence of 100 m*M* PLP. Each homogenate was assayed at least in triplicate. The ratio of induction by PLP was determined for each homogenate by dividing total cpm per milligram of protein per hour in the presence of PLP by the corresponding value in the absence of PLP. GAD activity in immunoprecipitated tissue homogenates was measured as above. Protein concentrations were determined by the Bradford colorimetric method (Bio-Rad) using bovine serum albumin as the standard (Bradford, 1976).

Western blots

Tissue homogenates or immunoprecipitated tissue samples were separated by electrophoresis on a 10% acrylamide-SDS gel and electrophoretically transferred to nitrocellulose using a Transblot cell (Bio-Rad) as previously described (Erlander et al., 1991). Blots were incubated with 2% bovine serum albumin (fraction V), 1% gelatin, and 1% Triton X-100 in phosphate-buffered saline for 1 h followed by primary antiserum K-2, which mainly recognizes GAD_{67} (Kaufman et al., 1991), or GAD-6, which only recognizes GAD_{65} (Chang and Gottlieb, 1988). For immunoblotting, all antisera were used at dilutions of 1:2,000. GAD-6 was used as a 1:2,000 dilution of ascites fluid. Filters were extensively washed and, in the case of GAD-6, incubated with rabbit anti-mouse IgG (Cappel). After the unbound secondary antibody was washed off, the filters were incubated with ^{125}I-protein A and exposed to Kodak XAR5 film.

Immunoprecipitation

Tissue homogenates (100 μl) were incubated at 0°C for 40 min with K-2 antiserum (2 μl) or with GAD-6 ascites fluid (0.1 μl), followed by incubation (20 min) with rabbit anti-mouse IgG for the GAD-6 antibody, and precipitated with CL-4B Sepharose–protein A beads (CL-4B Sepharose; Pharmacia) (Kaufman et al., 1991).

Immunohistochemistry

Adult rat females were anesthetized with pentobarbital sodium (Nembutal) and perfused through the aorta with 4% paraformaldehyde in 0.12 *M* phosphate buffer (pH 7.3). Oviducts were dissected and immersed in 20% sucrose overnight. Tissue was embedded in OCT compound and frozen on dry ice. Sections were cut at 10 μm using a cryostat microtome at −20°C, placed on gelatin-coated slides (room temperature), and stored at −70°C with desiccant for later use. Immunohistochemical detection was carried out as described by Kaufman et al. (1991). The sections were rinsed in 0.1 *M* Tris buffer containing 1% NaCl (wash buffer) and then blocked with normal goat serum diluted 1:30 for 1 h. K-2 antiserum and preimmune serum were used at 1:1,000, 1:2,000, and 1:4,000 dilutions. The tissue sections were incubated in the primary antiserum for ~4 h at room temperature and overnight at 4°C. Tissue sections were rinsed in wash buffer and incubated for 1 h in goat anti-rabbit IgG at 1:100 in 1% normal goat serum. After rinsing with wash buffer, the immunoreactivity was detected through the peroxidase–antiperoxidase method. Immunohistochemical immunoreactivity with GAD-6 monoclonal antibody was also detected through the peroxidase–antiperoxidase method, with 1:500 and 1:1,000 dilutions of GAD-6 and goat anti-mouse IgG at 1:75. Control sections were processed similarly, except for the substitution of preimmune rabbit serum or the omission of the GAD-6 antibody; controls showed no specific staining.

RESULTS

Both GAD_{67} and GAD_{65} RNAs are present in oviduct and testis

We first determined which peripheral tissues expressed GAD_{65} and GAD_{67} mRNAs. Of the 12 peripheral tissues examined, only oviduct and testis contained RNAs that hybridized to GAD_{67} and GAD_{65} cDNAs (Fig. 1). In contrast to the single 3.7-kb GAD_{67} RNA in brain, testis and oviduct expressed multiple species of GAD_{67} RNA (Fig. 1A). Testis contained six

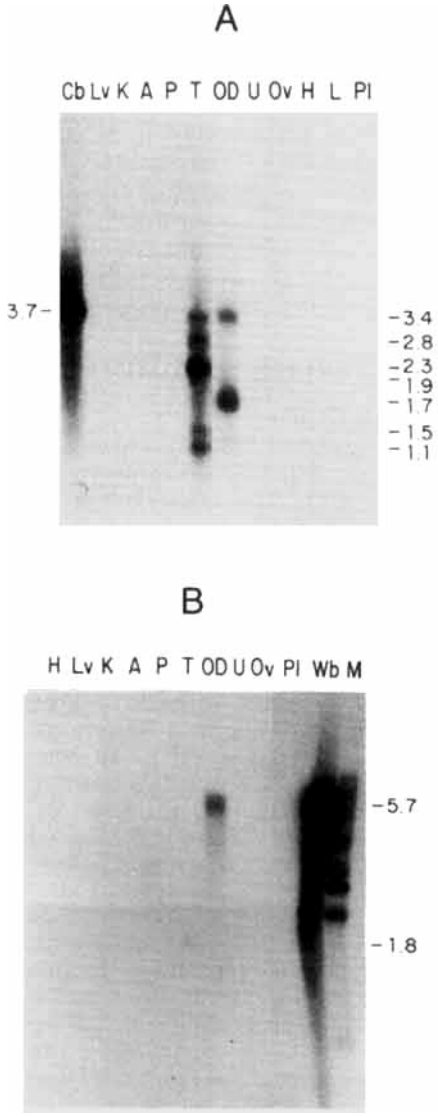

FIG. 1. Northern blots of RNA from rat tissues hybridized to (**A**) GAD_{67} and (**B**) GAD_{65} cDNA. Each lane contains 10 μg of poly(A)$^+$ RNA except that the cerebellum (Cb) lane contains 0.3 μg. Lv, liver; K, kidney; A, adrenal gland; P, pancreas; T, testis; OD, oviduct; U, uterus; Ov, ovary; H, heart; L, lung; Pl, placenta; Wb, whole brain; M, RNA ladder.

species of GAD_{67} RNA, whereas oviduct had two. In adult testis, GAD_{67} cDNA hybridized to RNAs of 3.4, 2.8, 2.3, 1.9, 1.5, and 1.1 kb. Oviduct contained 3.4- and 1.7-kb RNAs. Testis and oviduct thus shared only the 3.4-kb GAD_{67} RNA. GAD_{65} cDNA hybridized to a 5.7-kb RNA species in oviduct, the same size as GAD_{65} mRNA in brain, and to a rare 1.8-kb RNA in testis (Fig. 1B). Hybridization of both GAD_{65} and GAD_{67} probes persisted even at high stringency (0.1× SSC and 0.1% SDS at 72°C), a result showing strong sequence similarities among GAD RNAs in testis, oviduct, and brain.

GAD activity responds differently to PLP in oviduct and testis

GAD activity was present in homogenates of several tissues, at levels lower than in brain, including (in order of decreasing specific activity) kidney, oviduct, liver, testis, lung, ovary, pancreas, adrenal gland, and spleen (Table 1).

In all tissues except the oviduct and cerebellum, addition of PLP to the assay did not affect GAD activity. The stimulatory effect of PLP was greater in the oviduct (3.2-fold) than in the adult cerebellum (twofold). The increase of GAD activity with exogenously added PLP reflects the presence of apo-GAD (inactive GAD, no PLP bound), most of which is GAD_{65} (Martin, 1987; Erlander and Tobin, 1991; Kaufman et al., 1991). Of the GAD activity in oviduct, 87% was immunoprecipitated by GAD-6 antiserum, which is specific to GAD_{65} (Chang and Gottlieb, 1988), whereas only 12% was precipitated by K-2, which is specific to GAD_{67} (Kaufman et al., 1991). In testis, 30% of GAD activity was precipitated by K-2. No significant decrease of GAD activity was seen in any other tissues after immunoprecipitation using these two antibodies. Immunoprecipitation data thus support the conclusion that most of the GAD in oviduct is GAD_{65}, whereas testis GAD is predominantly GAD_{67}.

TABLE 1. *PLP dependence and immunoprecipitation of GAD activities in peripheral tissues*

Tissue	GAD Activity (nmol of CO_2/mg of protein/h)	Stimulation (fold) of GAD activity by added PLP	% of GAD activity precipitated by	
			K-2	GAD-6
Brain	219.2 ± 4.1	2.0	75	77
Oviduct	11.0 ± 0.16	3.2	12	86
Ovary	3.4 ± 0.05	1.2	10	8
Testis	6.2 ± 0.62	1.0	30	6
Liver	7.9 ± 0.45	1.1	9	6
Kidney	23.3 ± 3.4	1.1	5	2

K-2 and GAD-6 antiserum were used to immunoprecipitate selectively GAD_{67} or GAD_{65}, respectively, from tissue homogenates as described in Materials and Methods. The enzymatic activity of the remaining form of GAD in solution was examined with and without the addition of exogenous PLP cofactor. Data are mean ± SD values (n = 4).

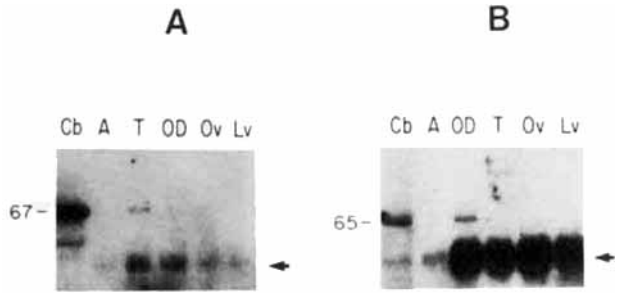

FIG. 2. Immunoblots of GAD_{67} and GAD_{65} proteins in peripheral tissues. Each lane except cerebellum (Cb) contains proteins immunoprecipitated with either (**A**) K-2 or (**B**) GAD-6 and detected with the same antibody. A, adrenal; T, testis; OD, oviduct; Ov, ovary; Lv, liver. The Cb lane contains 100 μg of protein of direct tissue homogenate. GAD_{67} immunoreactivity was seen in OD at longer exposures of the autoradiogram. The immunoreactivity seen below (indicated by arrows) GAD_{67} and GAD_{65} is due to immunoglobulins used in the immunoprecipitations. The bands that correspond to immunoglobulins are also present in the control immunoprecipitation reactions with preimmune sera.

The molecular size of GAD is 65,000 in oviduct and 67,000 in the testis

We did not detect immunoreactive GAD_{65} or GAD_{67} protein in extracts of liver, kidney, spleen, adrenal gland, pancreas, ovary, heart, and lung by western blot analysis (using tissue homogenates containing 100 μg of protein per lane) or after immunoprecipitation with K-2 and GAD-6. Rat oviduct contained mainly GAD_{65}, with low levels of GAD_{67} (Fig. 2). In contrast, testis contained only GAD_{67}. The sizes of the GAD_{65} and GAD_{67} in oviduct and testis are the same as in brain.

GAD_{65} and GAD_{67} RNAs and immunoreactivity is present in the mucosal epithelium of rat oviduct

We localized GAD and GAD mRNAs in the oviduct by immunohistochemistry and in situ hybridization. We observed both GAD_{65} and GAD_{67} immunoreactivity in the mucosal epithelium of the rat oviduct, with intense GAD_{65} immunoreactivity in the mucosal epithelial cells (Fig. 3). The ampulla (the part of the oviduct closer to ovary) contains more immunoreactive cells than the isthmus (closer to the uterus). Some GAD_{67} immunoreactivity was also seen in the cells of the oviductal epithelium.

In situ hybridization revealed that GAD_{65} RNAs are in the mucosal epithelium of the rat oviduct. Muscle and serosa layers of the oviduct did not contain either GAD RNA (Fig. 4). Mucosal epithelial cells also contained low levels of GAD_{67} RNA.

Germ line cells in testis contain GAD_{67} RNAs and GAD activity

To determine which cells in the testis contain GAD, we performed cell fractionation using unit gravity bovine serum albumin gradients. The purity of the cell populations isolated by this method was previously established (Collard et al., 1990). We then analyzed the

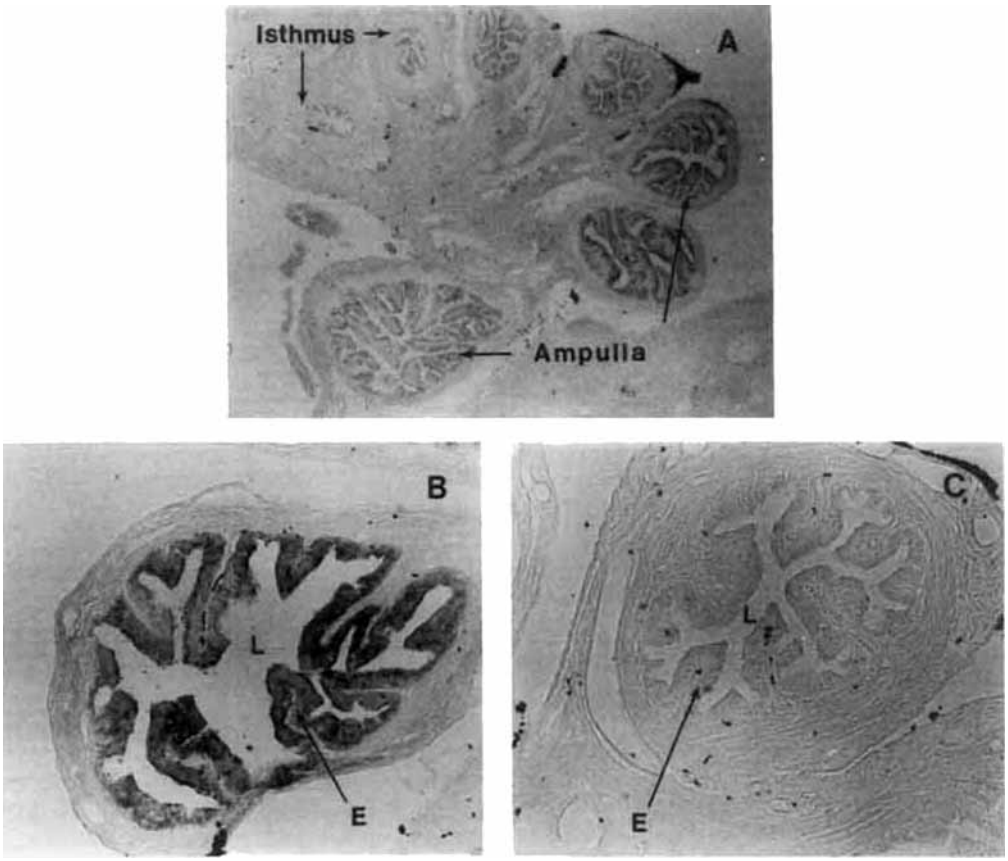

FIG. 3. GAD_{65} immunoreactivity in the mucosal epithelium of the rat oviduct. **A**: GAD_{65} immunoreactivity in various parts of the rat oviduct. More immunoreactivity is present in the oviductal ampulla than in the isthmus. **B**: GAD_{65} immunoreactivity is present in the epithelial cells (E) of the oviduct. In A and B, immunoreactivity was detected with GAD-6 antibody. **C**: Control.

separated cells for GAD activity and GAD mRNAs (by northern blotting).

Our data show that GAD_{67} RNAs are in germinal cells but not in somatic cells (Fig. 5). For example, GAD_{67} RNAs were present in total germinal cells, spermatocytes, and round spermatids but not in peritubular, Leydig, or Sertoli cells. Isolated germ cells also contained GAD activity (Table 2). As in the case of whole testis, addition of PLP to the assay did not affect the GAD activity. In situ hybridization to sections of testis showed GAD_{67} RNA associated with the seminiferous epithelium, with signal in spermatocytes and spermatids but not in interstitial cells (data not shown).

Levels of individual GAD_{67} RNAs change during postnatal testicular development

GAD_{67} RNAs were undetectable in the testes at 5 days after birth (Fig. 6). The first detectable GAD_{67} RNA in the testes was the 1.9-kb species, which appeared at postnatal day 10. The level of this species was highest at postnatal day 20 but remained prominent throughout the first 10 weeks of postnatal life. Other GAD RNAs (3.4, 2.8, and 2.3 kb) appeared at postnatal day 34. We first detected the 1.1-kb species at postnatal day 54 and the 1.5-kb species at postnatal day 72.

The relative proportions of individual GAD_{67} transcripts in older rats (6 months) were different from those of 72-day-old rats. The 2.3-kb GAD_{67} RNA was more prominent in older than in younger rats.

DISCUSSION

Rat oviduct GAD is primarily GAD_{65}

The sizes of the GAD polypeptides and mRNAs indicate that both brain GADs (GAD_{65} and GAD_{67}) are present in the oviduct, with GAD_{65} being the predominant form. Our findings are in agreement with many reports that GABA production in the rat oviduct depends on a GAD similar to that in brain. For example, biochemical properties, such as coenzyme requirement, thermosensitivity, substrate kinetics, and pH dependence of oviductal GAD, are similar to those of brain (Erdö et al., 1984). An antiserum to rat brain GAD (Oertel et al., 1981*b*) immunoprecipitated GAD activity in rat oviduct and posterior pituitary but not in liver, anterior pituitary, kidney, or ovary (Celotti et al., 1986). Using the same antiserum, Erdö et al. (1989) found GAD immunoreactivity in the mucosal epithelial cells of rat oviduct. The antiserum used in these studies, however, recognizes both GAD_{65} and GAD_{67} (Kaufman et al., 1986, 1991).

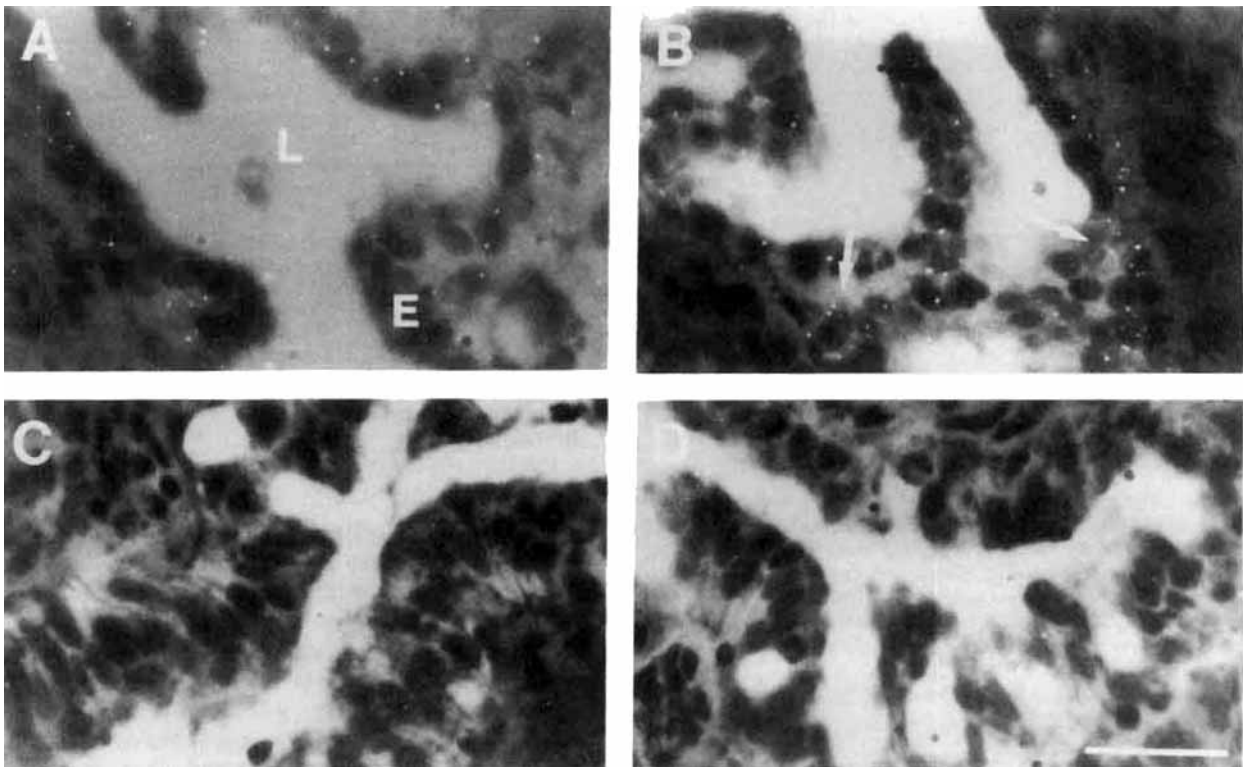

FIG. 4. Localization of GAD RNAs in the oviduct by in situ hybridization. Transverse sections of oviduct hybridized with ^{35}S-labeled GAD_{67} (A-anti-sense, C-sense) and GAD_{65} (B-anti-sense, D-sense). L, lumen of the oviduct; E, epithelial cells of the oviduct. Arrows indicate labeled epithelial cells. Bar = 10 μm.

Celloti et al. (1986) and Fernandez et al. (1985) have suggested that GABA and GAD in the rat oviduct may derive from GABA neurons, because denervation reduces both GABA and GAD activity. Immunocytochemistry reveals, however, that most of the GABA and GAD are not in neurons but in oviductal epithelium (Erdö et al., 1989). Our in situ hybridizations and immunohistochemical staining show that GAD_{65} and GAD_{67} are made in the mucosal epithelial cells of rat oviduct, a conclusion confirming the nonneural origin of GABA and GAD.

Rat testis contains GAD_{67}

In contrast to the oviduct, the testis does not contain PLP-dependent GAD activity. We detected strong hybridization of brain GAD_{67} cDNA to multiple RNA species in the testis, a finding suggesting that testis and brain GAD derive from the same gene. In fact, Persson et al. (1990) showed that a stretch of 69 amino acids derived from a GAD cDNA isolated from human testis using our feline GAD_{67} is identical to that of the rat brain.

Germ cells, including spermatocytes and round spermatids, contain GAD_{67} RNA, also in agreement with the results of Persson et al. (1990). These, as well as elongated spermatids, also contain GAD activity, an observation showing that the GAD RNAs make functional protein. The presence of elevated GAD activity but low GAD RNA levels in elongating spermatids suggests GAD RNAs are degraded after round spermatid development whereas GAD protein persists. We have observed a similar pattern of mRNA synthesis and protein production for proenkephalin (M. W. Collard, unpublished data).

The major species of GAD_{67} RNA in the brain is 3.7 kb long, whereas the longest GAD_{67} RNA in the testis is ~3.4 kb. Like GAD mRNA, the mRNAs encoding the opioid peptide precursors preproenkephalin, proopiomelanocortin, and dynorphin show different transcript sizes in brain and testis (Chen et al., 1984; Pintar et al., 1984; Kilpatrick et al., 1985). The multiple, testis-specific GAD_{67} RNAs may result from differential promoter usage or from differential splicing of a single GAD_{67} pre-mRNA.

We do not yet know which transcripts encode enzymatically active GAD_{67}. The 3.4-, 2.8-, 2.3-, and 1.9-kb species are long enough to encode a full-length (67,000-M_r) GAD polypeptide, but the 1.1- and 1.5-kb species are not.

GAD_{67} RNAs appear differentially during postnatal testicular development

Six distinct GAD_{67} RNAs are differentially expressed during the postnatal development of rat testis. All of these RNAs occur at elevated levels in isolated germ cells relative to whole testis or other testicular cell types.

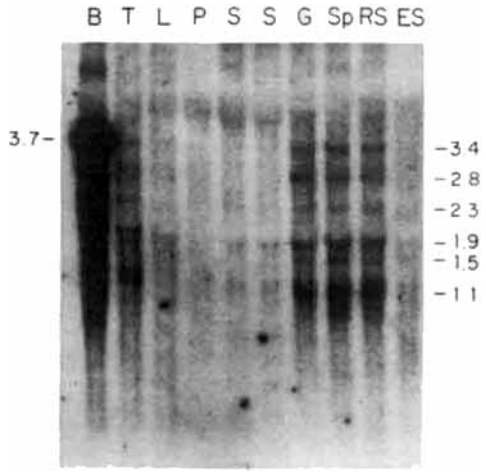

FIG. 5. Cellular localization of GAD_{67} RNAs in fractionated rat testicular cells. Each lane of this northern blot contains 10 μg of total RNA from brain (B), testis (T), acutely isolated Leydig cells (L), peritubular or myoid cells (P), Sertoli cells cultured in the absence (lane 5) and presence (lane 6) of 100 μM dibutyryl cAMP (S), total germinal cells (G), spermatocytes (Sp), round spermatids (RS), and elongating spermatids (ES). The blot was hybridized with a ^{32}P-labeled GAD_{67} antisense RNA probe.

The 1.9-kb GAD RNA, the first RNA species to appear, is detected at postnatal day 10, when it accounts for at least 0.003% of the poly(A)$^+$ RNA.

In addition to the 1.9-kb GAD RNA, several other GAD RNAs are sequentially expressed between postnatal days 34 and 72. During pubertal development, the 1.9-kb GAD RNA predominates. In the adult, however, the predominant GAD RNA is the 2.3-kb species. This developmental switch resembles that reported for the two forms of proenkephalin mRNA, which are also differentially regulated during the development of testis (Kilpatrick and Borland, 1987).

GAD_{67} RNAs are present at higher levels at postnatal days 54 and 72 than at postnatal day 34 and at their highest level in 6-month-old rats. Low levels of GAD RNAs in the prepubertal animals may be due to the low number of germ cells compared with the adults, because degeneration of germ cells occurs during the pubertal development of the rat (Russell et al., 1987). As development progresses, fewer germ cells degenerate. A progressive increase in content of GAD RNAs may result from this increased survival.

Function of GABA in oviduct and testis

The function of GABA and GAD in the oviduct and the testis is unknown. Erdö and Wolff (1990) suggested a morphogenetic role for GABA in the oviduct. This inference is based on the ability of benzodiazepines to block the migration of basal bodies during ciliogenesis in the oviduct (Boisvieux-Ulrich et al., 1987) and the appearance of GABA-like immunoreactivity in basal bodies of ciliated cells in the oviduct (Erdö et al., 1986).

GABA may also play a role in secretion in the oviduct. GAD_{65} is present at high levels in secretory cells

TABLE 2. *GAD activities in testicular cells*

Tissue	GAD activity (nmol of CO_2/mg of protein/h)	Stimulation (fold) of GAD activity by added PLP
Brain	219.2 ± 4.1	2.0
Spermatocytes	3.67 ± 0.66	0.95
Round spermatids	4.58 ± 0.61	1.0
Elongating spermatids	6.09 ± 0.16	1.0
TM-4 (Sertoli cell line)	2.5 ± 0.32	1.1
LC-540 (Leydig cell line)	0.23 ± 0.03	1.1

GAD activity in these homogenates was measured with and without the addition of exogenous PLP cofactor. Data are mean ± SD values (n = 4).

of the oviductal epithelium, which are also the predominant cells that accumulate GABA (Erdö and Amenta, 1986). Ovarian hormones, estrogen, and progesterone regulate the structural and biochemical characteristics of the oviduct (Karkun, 1979). Secretory and ciliary changes occur in the mucosal epithelium during the reproductive cycle. Significant changes in GABA and GAD levels also occur in the oviduct during the estrous cycle and gestation and after ovariectomy (Del Rio, 1981; Erdö, 1984; Fernandez-Pardal et al., 1984; Duvilanski et al., 1985; Celotti et al., 1986, 1987; Louzan et al., 1986; Murashima and Kato, 1986).

The presence of both forms of GAD in the oviduct may reflect different functional roles for each form. For example, one form may be involved in ciliary motility, whereas the other form is involved in secretory function. These forms may be subject to differential

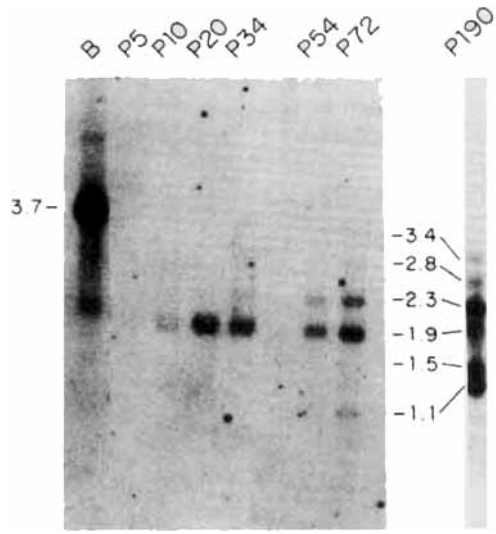

FIG. 6. GAD_{67} RNAs in postnatal testicular development. Each lane of the northern blot contains 3 μg of poly(A)$^+$ RNA from testes at different stages of postnatal development except the rat brain (B) lane, which contains 1 μg of poly(A)$^+$ RNA. Postnatal days 5, 10, 20, 34, 54, 72, and 190, P5, P10, P20, P34, P54, P72, and P190, respectively.

regulation by PLP, by other metabolites that may effect GAD–PLP interactions, or by ovarian hormones themselves.

GABA may also participate in the endocrine function in testis. GABA stimulates the production of testosterone in whole decapsulated testis (Ritta et al., 1987) and in testicular interstitial cell membranes (Ritta et al., 1991). Binding assays show the presence of $GABA_A$ and $GABA_B$ receptors in testicular intertitial cells and purified Leydig cells (Ritta et al., 1991). We find mRNAs of the α_1 subunit of the $GABA_A$ receptor expressed in the Leydig cell line LC-540 (N. J. K. Tillakaratne, unpublished data). GABA may also have a direct effect on spermatozoa because $GABA_A$-type binding sites are present on seminal membranes (Erdö and Wekerle, 1990). Persson et al. (1990) suggested GABA as an energy source for flagellar action. This is based on presence of GAD-like immunoreactivity in the midpiece of spermatozoa, which also contains a sheaf of mitochondria. In support of this idea, sodium valproate (an inhibitor of GAD and GABA transaminase) decreases sperm number and sperm motility in rats (Cohn et al., 1982).

Are GADs in nonneural tissues the same as brain GADs?

Oviduct and testis contain GADs that are similar to those in brain both with respect to common epitopes and common coding sequences as detected by high-stringency hybridization. If the nonneural GADs are similar to the two brain GAD forms (GAD_{67} and GAD_{65}), one would expect to see a similarity between GAD RNAs or proteins. We did not detect any GAD RNAs or proteins to both forms of GAD in liver, kidney, heart, adrenal gland, uterus, intestine, lung, or pancreas. GAD forms similar to that in brain may be present only in a few specialized cells in these peripheral tissues, thus being undetected when whole organs or tissues are assayed. This is certainly the case for β cells in the pancreas, where this laboratory has shown immunoreactivity to both GAD_{65} and GAD_{67} forms (D. L. Kaufman et al., manuscript submitted; N. J. K. Tillakaratne, unpublished data). GAD activity in liver and kidney is higher than that of the oviduct and testis. The presence of GABA and GAD is well documented in isolated hepatocytes (Minuk, 1986) and in kidney tubules (Goodyer et al., 1980, 1982). GAD activity in these nonneural tissues might derive from other GAD forms, such as those reported by Wu et al. (1986*a*), Denner et al. (1987), and Huang et al. (1990).

The molecular biological, immunological, and enzymological data presented here show that GAD_{65} and GAD_{67}, at least, are present in a restricted set of nonneural cells. Our results thus lend strong support for the suggestion that GABA plays a physiological role other than neurotransmission.

Acknowledgment: We are grateful to Carolyn Houser and Barbara Tibbs for helping us with immunohistochemistry. We also thank Dona Chikaraishi for the rat brain cDNA library and David Gottlieb for providing us with the GAD-6 monoclonal antibody. We are grateful to the following people for helpful discussions: Bu Dingfang, Sophie Feldblum, Gretchen Frantz, Daniel Kaufman, Helen Kim, George Lawless, Lali Medina-Kauwe, Cathy Pinal, Christian Ruppert, Jose Segovia, and Robert Weatherwax. This work was supported by grants NS 20356 and 22256 to A. J. Tobin from the NINCDS. N. J. K. Tillakaratne was partially supported by fellowships from the American Association of University Women and Phi Beta Kappa.

REFERENCES

Apud J. A., Tappaz M. L., Celotti F., Negri-Cesi P., Masotto C., and Racagni G. (1984) Biochemical and immunochemical studies on the GABAergic system in the rat fallopian tube and ovary. *J. Neurochem.* **43,** 120–125.

Baekkeskov S., Aanstoot H., Christgaus S., Reetz A., Solimena M., Cascalho M., Folli F., Richter-Oleson H., and DeCamilli P. (1990) Identification of the 64K auto-antigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. *Nature* **347,** 151–156.

Boisvieux-Ulrich E., Laine M.-C., and Sandoz D. (1987) In vitro effects of benzodiazapines on ciliogenesis in the quail oviduct. *Cell Motil. Cytoskeleton* **8,** 333–344.

Bradford M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72,** 248–254.

Celloti F., Apud J. A., Rovescalli A. C., Melcangi R. C., Negri-Cesi P., and Racagni G. (1986) The GABAergic extrinsic innervation of the rat fallopian tubes: biochemical evidence and endocrine modulation, in *Advances in Biochemical Psychopharmacology, Vol. 42: GABA and Endocrine Function* (Racagni G., and Donoso A. O., eds), pp. 251–264. Raven Press, New York.

Celloti F., Apud J. A., Rovescalli A. C., Negri-Cesi P., and Racagni G. (1987) Possible involvement of ovarian mechanisms other than estrogen-progesterone secretion in the regulation of glutamic acid decarboxylase activity of the rat fallopian tubes. *Endocrinology* **120,** 700–706.

Chang Y.-C. and Gottlieb D. I. (1988) Characterization of the proteins purified with monoclonal antibodies to glutamic acid decarboxylase. *J. Neurosci.* **8,** 2123–2130.

Chen C. C., Mather J. P., Morris P. L., and Bardin C. W. (1984) Expression of pro-opiomelanocortin-like gene in the testis and epididymis. *Proc. Natl. Acad. Sci. USA* **81,** 5672–5675.

Chirgwin J. M., Przybyla A. E., MacDonald R. J., and Rutter W. J. (1979) Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* **18,** 5294–5299.

Church G. M. and Gilbert W. (1984) Genomic sequencing. *Proc. Natl. Acad. Sci. USA* **81,** 1991–1995.

Cohn D. F., Homonnai Z. T., and Paz G. F. (1982) The effect of anticonvulsant drugs on the development of male rats and their fertility. *J. Neurol. Neurosurg. Psychiatry* **45,** 844–846.

Collard M. W., Day R., Akil H., Uhler M. D., and Douglass J. O. (1990) Sertoli cells are the primary site of prodynorphin gene expression in rat testis: regulation of mRNA synthesis and peptide secretion by cAMP analogs in cultured cells. *Mol. Endocrinol.* **4,** 1488–1496.

Del Rio R. M. (1981) γ-Aminobutyric acid system in rat oviduct. *J. Biol. Chem.* **256,** 9816–9819.

Denner L. A. and Wu J.-Y. (1985) Two forms of rat brain glutamic acid decarboxylase differ in their dependence on free pyridoxal phosphate. *J. Neurochem.* **44,** 957–965.

Denner L. A., Wei S. C., Lin H. S., Lin C.-T., and Wu J-Y. (1987) Brain L-glutamate decarboxylase: purification and subunit structure. *Proc. Natl. Acad. Sci. USA* **84,** 668–672.

Dorrington J. H. and Fritz I. B. (1975) Cellular localization of 5-alpha-reductase and 3-alpha-hydroxysteroid dehydrogenase in the seminiferous tubule of the rat testis. *Endocrinology* **96,** 879–889.

Duvilanski B. H., del Carmen Diaz M. C., Lasaga M., and Seilicovich A. (1985) L-Glutamate decarboxylase activity in the ovary and fallopian tube during the estrous cycle. *IRCS Med. Sci. (Biochem.)* **13,** 81.

Erdö S. L. (1984) Alteration of GABA levels in ovary and fallopian tube of the pregnant rat. *Life Sci.* **34,** 1879–1884.

Erdö S. L. and Amenta F. (1986) Characterization and localization of high-affinity GABA uptake in rabbit oviduct. *Eur. J. Pharmacol.* **130,** 287–294.

Erdö S. L. and Kiss B. (1986) Presence of GABA, glutamate decarboxylase, and GABA transaminase in peripheral tissues: a collection of quantitative data, in *GABAergic Mechanisms in the Mammalian Periphery* (Erdö S. L. and Bowery N. G., eds), pp. 5–17. Raven Press, New York.

Erdö S. L. and Wekerle L. (1990) $GABA_A$ type binding sites on membranes of spermatozoa. *Life Sci.* **47,** 1147–1151.

Erdö S. L. and Wolff J. R. (1990) γ-Aminobutyric acid outside the mammalian brain. *J. Neurochem.* **54,** 363–372.

Erdö S. L., Kiss B., and Szporny L. (1984) Comparative characterization of glutamate decarboxylase in crude homogenates of oviduct, ovary, and hypothalamus. *J. Neurochem.* **43,** 1532–1537.

Erdö S. L., Somogyi J., Hamori J., and Amenta F. (1986) Light- and electron-microscopic visualization of gamma-aminobutyric acid and GABA-trans-aminase in the oviduct of rats. Predominant occurrence in epithelium. *Cell Tissue Res.* **244,** 621–624.

Erdö S. L., Joó F., and Wolff J. R. (1989) Immunohistochemical localization of glutamate decarboxylase in the rat oviduct and ovary: further evidence for non-neural GABA systems. *Cell Tissue Res.* **255,** 431–434.

Erlander M. G. and Tobin A. J. (1991) The structural and functional heterogeneity of glutamate decarboxylase: a review. *Neurochem. Res.* **16,** 215–226.

Erlander M. G., Tillakaratne N. J. K., Feldblum S., Patel N., and Tobin A. J. (1991) Two genes encode distinct glutamate decarboxylases with different responses to pyridoxal phosphate. *Neuron* **7,** 91–100.

Feinberg A. P. and Vogelstein B. (1984) Addendum: a technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* **137,** 266–267.

Fernandez I., Martin del Rio R., and Orensanz L. M. (1985) Surgical ablation of oviductal extrinsic innervation changes GABA levels in the rat Fallopian tube. *Life Sci.* **36,** 1733–1737.

Fernandez-Pardal J., Gimeno M. F., and Gimeno A. L. (1984) The presence of GABA in ovarian, tubal and uterine rat tissue and the influence of estrogens. *IRCS Med. Sci. (Biochem.)* **12,** 706.

Goodyer P. R., Lancaster G., Villeneure M., and Scriver C. R. (1980) The relationship of 4-aminobutyric acid metabolism to ammoniagenesis in renal cortex. *Biochim. Biophys. Acta* **633,** 191–200.

Goodyer P. R., Mills M., and Scriver C. R. (1982) Properties of gamma-aminobutyric acid synthesis by rat renal cortex. *Biochim. Biophys. Acta* **716,** 348–357.

Huang W.-M., Reed-Fourguet L., Wu E., and Wu J.-Y. (1990) Molecular cloning and amino acid sequence of brain L-glutamate decarboxylase. *Proc. Natl. Acad. Sci. USA* **87,** 8491–8495.

Jessen K. R., Mirsky R., Dennison M. E., and Burnstock G. (1979) GABA may be a neurotransmitter in the vertebrate peripheral nervous system. *Nature* **281,** 71–74.

Karkun J. V. (1979) Recent advances on the endocrinology of the mammalian oviduct, in *Recent Advances in Reproduction and Regulation of Fertility* (Talwar G. P., ed), pp. 189–199. Elsevier/North Holland Biomedical Press, Amsterdam.

Kataoka Y., Gutman Y., Guidotti A., Panula P., Wroblewski J., Cosenza-Murphy D., Wu J. Y., and Costa E. (1984) Instrinsic GABAergic system of adrenal chromaffin cells. *Proc. Natl. Acad. Sci. USA* **81,** 3218–3222.

Kaufman D. L., McGinnis J. F., Krieger N. R., and Tobin A. J. (1986) Brain glutamate decarboxylase cloned in λ-gt-11: fusion protein produces γ-aminobutyric acid. *Science* **232,** 1138–1140.

Kaufman D. L., Houser C. R., and Tobin A. J. (1991) Two forms of the GABA synthetic enzyme glutamate decarboxylase have distinct intraneuronal distributions and cofactor interactions. *J. Neurochem.* **56,** 720–723.

Khrestchatisky M., MacLennan A. J., Tillakaratne N. J. K., Chiang M.-Y., and Tobin A. J. (1991) Sequence and regional distribution of the mRNA encoding the α2 polypeptide of rat γ-aminobutyric $acid_A$ receptors. *J. Neurochem.* **56,** 1717–1722.

Kilpatrick D. L. and Borland K. (1987) Differential expression of two distinct forms of proenkephalin mRNA during development of the mouse testis. *Ann. N. Y. Acad. Sci.* **15,** 315–317.

Kilpatrick D. L., Howells R. D., Noe M., Bailey C. L., and Udenfriend S. (1985) Expression of preproenkephalin-like mRNA and its peptide products in mammalian testis and ovary. *Proc. Natl. Acad. Sci. USA* **82,** 7467–7469.

Kobayashi Y., Kaufman D. L., and Tobin A. J. (1987) Glutamic acid decarboxylase cDNA: nucleotide sequence encoding an enzymatically active protein. *J. Neurosci.* **7,** 2768–2772.

Krieger N. R. and Heller J. S. (1984) Localization of GAD within laminae of rat olfactory tubercle. *J. Neurochem.* **33,** 299–309.

Legay F., Henry S., and Tappaz M. (1987) Evidence for two distinct forms of native glutamic acid decarboxylase in rat brain soluble extract: an immunoblotting study. *J. Neurochem.* **48,** 1022–1026.

Louzan P., Gallardo M. G. P., and Tramezzani J. H. (1986) Changes of GABA content in the female rat genital tract, in *GABA and Endocrine Function* (Racagni G. and Donoso A. O., eds), pp. 283–290. Raven Press, New York.

Maniatis T., Fritsch E. F., and Sambrook J. (1982) *Molecular Cloning. A Laboratory Manual,* p. 202. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Martin D. L. (1987) Regulatory properties of brain glutamate decarboxylase. *Cell. Mol. Neurobiol.* **7,** 237–253.

Meistrish M. L., Bruce W. R., and Clermont Y. (1973) Cellular composition of mouse testis cells following velocity sedimentation separation. *Exp. Cell Res.* **79,** 213–227.

Minuk G. Y. (1986) GABAergic mechanisms and their functional importance in the liver, in *GABAergic Mechanisms in the Mammalian Periphery* (Erdö S. L. and Bowery N. G., eds), pp. 325–337. Raven Press, New York.

Morales C., Hugly S., and Griswold M. D. (1987) Stage-dependent levels of specific mRNA transcripts in Sertoli cells. *Biol. Reprod.* **36,** 1035–1046.

Murashima Y. L. and Kato T. (1986) Distribution of γ-aminobutyric acid and glutamate decarboxylase in the layers of rat oviduct. *J. Neurochem.* **46,** 166–172.

Oertel W. H., Schmechel D. E., Weise V. K., Ransom D. H., Tappaz M. L., Krutzch H. C., and Kopin I. J. (1981*a*) Comparison of cysteine sulphinic acid decarboxylase isozymes and glutamic acid decarboxylase in rat liver and brain. *Neuroscience* **6,** 2701–2714.

Oertel W. H., Schmechel D. E., Tappaz M. L., and Kopin I. J. (1981*b*) Production of a specific antiserum to rat brain glutamic acid decarboxylase by injection of an antigen-antibody complex. *Neuroscience* **6,** 2689–2700.

Persson H., Delto-Huikko M., Metsis M., Söder O., Stefan B., Skog S., Hökfelt T., and Ritzén E. M. (1990) Expression of the neurotransmitter-synthesizing enzyme glutamic acid decarboxylase in male germ cells. *Mol. Cell. Biol.* **10,** 4701–4711.

Pintar J. E., Schachter B. S., Herman A. B., Durgerian S., and Krieger D. T. (1984) Characterization and localization of proopiomelanocortin messenger RNA in the adult testis. *Science* **225,** 632–634.

Redburn D. A. and Schousboe A., eds (1987) *Neurotrophic Activity of GABA During Development.* Alan R. Liss, New York.

Ritta M. N., Campos M. B., and Calandra R. S. (1987) Effect of GABA and benzodiazepines on testicular androgen production. *Life Sci.* **40,** 791–798.

Ritta M. N., Campos M. S., and Calandra R. S. (1991) Coexistence of γ-aminobutyric Acid type A and type B receptors in testicular interstitial cells. *J. Neurochem.* **56,** 1236–1240.

Rorsman P., Berggre P.-O., Bokvist K., Ericson H., Mohler H., Ostenson C.-G., and Smith P. A. (1989) Glucose-inhibition of glucagon secretion involves activation of $GABA_A$ receptor chloride channels. *Nature* **341,** 233–236.

Russell L. D., Alger L. E., and Neguin L. G. (1987) Hormonal control

of pubertal spermatogenesis. *Endocrinology* **120,** 1615–1632.

Segovia J., Tillakaratne N. J. K., Whelan K., Tobin A. J., and Gale K. (1990) Parallel increases in striatal glutamic acid decarboxylase activity and mRNA levels in rats with lesions of the nigrostriatal pathway. *Brain Res.* **529,** 345–348.

Spink D. C., Porter T. G., Wu S. J., and Martin D. L. (1985) Characterization of three kinetically distinct forms of glutamate decarboxylase from pig brain. *Biochem. J.* **231,** 695–703.

Tillakaratne N. J. K. and Tobin A. J. (1986) Glutamic acid decarboxylase (GAD) messenger RNA in brain and peripheral tissues of rat. (Abstr) *Soc. Neurosci. Abstr.* **12,** 1458.

Tobin A. J. (1989) Structure and regulation of the gene for the glutamate decarboxylase: a candidate gene for neuropsychiatric and neurological disease, in *Molecular Neurobiology, Proceedings of the First NIMH Conference* (Zalcman S. and Scheller R., eds), pp. 165–182. U.S. Department of Health and Human Services, Rockville, Maryland.

Vincent S. R., Hökfelt T., Wu J.-Y., Elde R. P., Morgan L. M., and Kimmel J. R. (1983) Immunohistochemical studies of the GABA system in the pancreas. *Neuroendocrinology* **36,** 197–204.

Whelan D. T., Scriver C. R., and Mohyuddin F. (1969) Glutamic acid decarboxylases and gamma-aminobutyric acid in mammalian kidney. *Nature* **224,** 916–917.

Wood T. L., Frantz G. D., Menkes J. H., and Tobin A. J. (1986) Regional distribution of messenger RNAs in postmortem human brain. *J. Neurosci. Res.* **16,** 311–324.

Wu J.-Y. (1977) A comparative study of L-glutamate decarboxylase from mouse brain and bovine heart with purified preparations. *J. Neurochem.* **28,** 1359–1367.

Wu J.-Y. and Roberts E. (1973) Comparative studies of L-glutamate decarboxylases from mouse brain and kidney. (Abstr) *Am. Soc. Neurochem.* **4,** 70.

Wu J.-Y., Chude O., Wein J., and Roberts E. (1978) Glutamate decarboxylase from neural and non-neural tissues. *J. Neurochem.* **30,** 849–857.

Wu J.-Y., Denner L. A., Wei S. C., Lin C.-T., Song G.-X., Xu Y. F., Liu J. W., and Lin H. S. (1986*a*) Production and characterization of polyclonal and monoclonal antibodies to rat brain L-glutamate decarboxylase. *Brain Res.* **373,** 1–14.

Wu J.-Y., Lin C.-T., Lin H., Xu Y., Liu J. W., Hwang B. H., and Wei S. C. (1986*b*) Immunochemical characterization and immunohistochemical localization of glutamate decarboxylase and GABA transaminase in peripheral tissues, in *GABAergic Mechanisms in the Mammalian Periphery* (Erdö S. L. and Bowery N. G., eds), pp. 19–34. Raven Press, New York.

Wuenschell C. W., Fisher R. S., Kaufman D. L., and Tobin A. J. (1986) *In situ* hybridization to localize mRNA encoding the neurotransmitter synthetic enzyme glutamate decarboxylase in mouse cerebellum. *Proc. Natl. Acad. Sci. USA* **83,** 6193–6197.