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Gabaergic and cholinergic systems of the oviduct

Sistema gabaérgico y colinérgico del oviducto

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The rat oviduct contains a GABAergic system with biochemical and immunological characteristics similar to those present in the brain. However, recent immunohistochemical studies indicate that the GABAergic machinery is localized only in the mucosa and not in the nerve terminals.

Ovarian sex hormones, when given in high doses, are able to affect GAD activity of the oviducts, however the possible modulatory role of these hormones in physiological situations is less well defined.

The role of the GABAergic system in the mucosa of the oviducts is presently unknown, but a paracrine role in the modulation of oviductal, ovarian or uterine functions may be proposed.

Acetylcholine stimulates oviductal muscle contraction apparently by the postganglionic release of norepinephrine. However a direct cholinergic mechanism has been shown to interact with sex steroids in regulating GAD activity.

INTRODUCTION

The fallopian tubes, due to their importance in the reproductive process, are kept under the control of endocrine, paracrine and nervous physiological mechanisms. Not all these mechanisms and their possible interactions have been thoroughly investigated so far.

Here, we review the most relevant evidence for the existence of a GABAergic system in the rat oviduct; moreover, rhe few available data on the presence of the cholinergic system in the tubes will be summarized.

GABAergic system

Since 1950, it has been known that large amounts of γ aminobutyric acid (GABA) are present in the central nervous system of vertebrates (Roberts and Frankel, 1950), where it acts mainly as an inhibitory neurotransmitter; in more recent years, GABA has also been found both in the peripheral autonomic innervation and in several tissues and endocrine glands of many animal species (Tanaka, 1985). In particular, GABA has been shown to be the inhibitory neurotransmitter of the peripheral motor nerves of invertebrates (Kravitz *et al.*, 1965) and significant amounts of GABA have been found in neurons of the myenteric plexus, where it participates in the regulation of intestinal peristalsis (Jessen *et al.*, 1979; Miki *et al.*, 1983). Among non-neuronal tissues, GABA has been found in high concentration in the kidney (Zachmann *et al.*, 1966), in the pancreatic β-cells (Okada *et al.*, 1976; Gerber and Hare, 1979; Taniguchi *et al.*, 1979), in the oviduct (see below) and, although at a minor extent, in the adrenal glands and in the ovary (Tanaka, 1985). In spite of this wide distribution, little is known of the exact localization and the physiological functions of GABA in non-neural tissues. In these, GABA might be present in the nerve terminals, acting as a neurotransmitter, or in secretory cells having a paracrine and/or autocrine role. Up to now, the presence of an extrinsic GABAergic innervation in peripheral tissues, has been clearly demonstrated only in the gut (Jessen *et al.*, 1987). It has been suggested to be present also in the oviduct; however, no direct morphological evidence of the exis-

tence of GABAergic nerve endings in the oviduct has been obtained so far. Nevertheless, the presence of several elements of the biochemical machinery usually associated with GABA synthesis and turnover, as well as the results obtained in experiments of grafting and denervation, have led to hypothesize the existence of an extrinsic GABAergic innervation of this organ. The biochemical components possibly associated with the presence of a GABAergic innervation are summarized in table 1.

The first report on the presence of GABA in the female reproductive system (Martín del Río and Latorre Caballero, 1980), described the presence of relatively high levels of GABA in the ovaries of rats. Soon thereafter it was realized (Martín del Río, 1981) that it was not the ovary but the oviduct, clearly not removed or incompletely removed in those initial experiments, the organ that contained high levels of GABA.

Table 2 shows the results obtained in our laboratory (Apud *et al.*, 1984) which indicate that the GABA content of the oviducts, obtained from normal diestrous female rats, is twice as high as in the brain; while in the ovary, GABA levels are extremely low (about 4.9% of the brain concentration of the aminoacid). The oviducts possess glutamic acid decarboxylase (GAD; EC 4.1.1.15) activity, the key enzyme in GABA synthesis (about 12% of that present in the brain), while the ovaries possess a much smaller amount of this enzymatic activity (about 1% of that of brain).

TABLE 1

Biochemical components possibly associated with a GABAergic innervation

- Presence of high concentrations of GABA.
- Presence of glutamic acid decarboxylase (GAD), the key enzyme for GABA synthesis.
- Biochemical characteristics of oviductal GAD analogous to those of neuronal GAD.
- Cross-reactivity of oviductal GAD with specific antisera directed against neuronal GAD.
- Presence of GABA transaminase (GABA-T), the enzyme responsible for GABA catabolism.
- Presence of a GABA uptake system.
- Presence of high affinity GABA binding sites.
- Decrease of GABA levels and GAD activity after oviductal denervation or transplantation.

TABLE 2

GABA levels and GAD activity in the brain ovary and oviduct of diestrous rats

	GABA (nmol/mg prot)	GAD ($nmolCO_2$/mg prot/hr)
Brain	20.5±1.3 (15)	230.0±16.0 (15)
Ovary	1.0±1.0 (28)	2.5± 0.2 (15)
Oviduct	46.0±3.6 (14)	27.7± 1.6 (15)

Values are means ± SEM; numbers of animals in parenthesis.

In this set of experiments, all the tissues utilized to evaluate GABA content were subjected to a focal microwave irradiation (2.0 kW 2.45 GHz/cm^2) immediately after surgical collection (usually within 2 minutes after killing the animals), in order to avoid the nonspecific post-mortem increase in GABA concentration which seems to occur also in the peripheral tissues, even if this increase is probably not quantitatively as important and as rapid as that occuring in the CNS (Guidotti *et al.*, 1974; Apud *et al.*, 1984). GABA was evaluated by a specific and sensitive gas chromatographic-mass fragmentografic method (Cattabeni *et al.*, 1976).

GAD activity was measured evaluating the formation of labelled CO_2 during an incubation of supernatants from the tissue homogenates with carbon-labelled glutamate under anaerobic condition as described elsewhere (Apud *et al.*, 1984).

GABA contents and GAD activities comparable to those found in the studies just described have been reported by different authors (Martín del Río, 1981; Erdö *et al.*, 1982, 1984a, 1986a; Fernández-Pardal *et al.*, 1984).

Since it is known that in peripheral tissues carbon dioxide can be formed by other metabolic pathways (*e.g.* the deamination of glutamic acid to α-ketoglutarate in the Krebs cycle) (Drummond and Phillis, 1974), non specific CO_2 formation might lead to overestimations or erroneous results, particularly in structures with low endogenous GAD. Therefore, it was decided to measure the GAD activity utilizing simultaneously both the CO_2 evolution, as previously described, and the [^{14}C] GABA formation from uniformly labelled glutamic acid in the high speed supernatant of several tissues (Apud *et al.*, 1984).

The results of these experiments indicated that detectable amounts of carbon dioxide are formed by all the tissues tested (anterior and posterior pituitary, oviducts, kidney and liver); however, labelled GABA is formed only by the posterior pituitary included as control tissue since it possess a GABAergic innervation of central origin) (Oërtel *et al.*, 1982), the oviduct and the kidney (6, 5 and 1% of the brain GAD activity respectively). On the contrary GABA formation in the ovary, in the anterior pituitary and in the liver is under the detection limit of the method. Moreover, a good correlation between the two methods used seems to exist for the posterior pituitary and for the oviduct, even if the carbon dioxide formation gives a small overestimation of the enzymatic activity. These results are in substantial agreement with those obtained in contemporary studies by Erdö and coworkers (1984a) who found that stoichiometric formation of GABA and CO_2 can be observed only in the oviduct and in the hypothalamus and not in the ovary. It appears therefore from these results, that among the peripheral tissues tested, only the oviduct is able to produce a substantial amount of GABA from glutamate. To clarify whether the GAD detected in this structure is the same enzyme present in the brain, immunoprecipitation experiments were performed using an antibody raised in the sheep and specifically directed against rat neuronal GAD (Oërtel *et al.*, 1981). The GAD activity was measured as labelled carbon dioxide formation after a preincubation period with or without the antiserum. In this experiment as well as in those to be described below, the CO_2 evolution method was selected instead of the labelled GABA formation, even if it gives a small overestimation of the enzymatic activity, because it is more sensitive and easy to perform. Among the tissues considered (anterior and posterior pituitary, oviduct, kidney and liver) only in the posterior pituitary and in the oviduct, GAD activity was quantitatively decreased after immunoprecipitation; carbon dioxide formation in the liver, ovary, anterior pituitary and kidney remained substantially unaffected by the preincubation with the antiserum. It seems therefore that the oviduct is the only peripheral organ equipped with an enzyme immunologically similar to the one found in the central nervous system.

Further evidence of the similarity between the oviductal and cerebral GAD, comes from a series of experiments by Erdö and coworkers (1984a). These authors evaluated some biochemical parameters (such as the time course of the reaction, the linearity with the protein concentration, the pH and temperature dependence and the coenzyme requirements) and confirmed the biochemical similarity between the oviductal and the hypothalamic GAD. Moreover, the Michaelis-Menten constant (K_m), an index of the affinity of a substrate for an enzyme, is very similar for the GAD present in the two tissues; while the V_{max}, which indicates the amount of enzyme present, is obviously much higher for the hypothalamus than for the oviduct. Also the behavior of the brain and oviductal enzymes in the presence of competitive and non-competitive inhibitors is analogous.

The oviduct not only possesses the enzyme involved in GABA synthesis, but also GABA-transaminase (GABA-T) *i.e.* the enzyme which provides the major degradation pathway for GABA. This enzyme was found in crude homogenates of rat (Martín del Río, 1981; Erdö *et al.*, 1982; 1984a; 1986a) and mouse (Wu, 1982) oviduct, even if its activity seems to be less than one half of that present in the brain. However, since the enzymatic activity cannot be fully blocked by specific inhibitors, such as aminooxyacetic acid, gabaculine or ethanolamine-o-sulphate (Erdö *et al.*, 1982; Martín del Río and López, 1983; Apud *et al.* unpublished results) administered either *in vivo* or *in vitro*, the involvement of other oxygen-dependent catabolic patways has been postulated.

Extremely low GABA uptake and release mechanisms have been found in the rat oviduct by Erdö (1986a). Since other authors were unable to confirm these data (Orensanz *et al.*, 1986, Apud *et al.*, unpublished observations), the presence of such

mechanisms in the organ deserves further investigation.

The data on the presence of specific GABA-binding sites in the oviduct are still controversial (see Table 3): Erdö's group identified GABA receptors in both rat and human membrane preparations; the kinetic parameters appear to be consistent with a single population of high-affinity (K_d in the nanomolar range) GABA-A receptors (Erdö and Lapis, 1982; Erdö *et al.*, 1983; Erdö, 1986b) similar to those present in the brain. The type A nature of the oviductal GABA receptors has also been confirmed in displacement experiments by the same authors: tritium labelled GABA could be displaced from the oviductal binding sites by either muscimol or bicuculline, two compounds known to be specific ligands for GABA-A receptors (Erdö and Lapis, 1982; Erdö *et al.*, 1983). Moreover, Erdö and Maksay (1988) demonstrated that labelled TBPS (a convulsant which is a specific ligand for the GABA-A receptor coupled chloride ionophore) binds to tubal membranes and that both GABA and penthobarbital are able to modulate the TBPS binding in a fashion similar to that observed in the brain. Under conditions typical for a GABA-B receptor assay, it was found that labelled baclofen (a selective ligand of GABA-B receptors) displays some specific binding to isolated rabbit oviductal membranes (Erdö *et al.*, 1984b); however, the low level of specific binding, not sufficient to characterize these binding sites, does not allow to confirm the presence of such type of receptors in the oviduct. Evidence for the existence of either GABA-A or GABA-B receptors in the oviduct could not be reproduced by other groups (Fernández *et al.*, 1981; Orensenz and Fernández, 1985), including ourselves (Apud, unpublished observations). Therefore the actual existence of specific GABA receptors in the oviduct deserves further confirmation.

TABLE 3

GABA binding sites in the oviduct

Tissue preparation	Labelled ligand	K_d (nM)	V_{max} (fmol/ mg prot)	Characteristic	Reference
Rat membranes	GABA	52	17	Displaced by muscimol and bicuculline GABA-A receptor	Erdö and Lapis, 1982
Rat membranes	GABA	52	117	Improved membrane preparation GABA-A receptor	Erdö, 1986b
Human membranes	GABA	40	690	Displaced by muscimol but not by baclofen probably GABA-A receptor	Erdö *et al.*, 1983
Rat membranes	TBPS	–	–	TBPS binding sites allosterically modulated by GABA and pentobarbital GABA-A receptor	Erdö and Maksay, 1988
Rat membranes	GABA	–	–	Low proportion of GABA-B specific receptors	Erdö *et al.*, 1948b
Rat membranes	GABA	–	–	No specific binding sites	Fernández *et al.*, 1981
Rat membranes	GABA	–	–	No specific binding sites	Orensanz and Fernández, 1985
Rat membranes	GABA Muscimol	– –	– –	No specific binding sites	Apud *et al.*, unpubl.

In an attempt to further clarify whether the GABAergic system of the oviduct comes from the peripheral innervation, transplantation or denervation experiments were performed in our laboratory (Apud *et al.*, 1984). In brief, adult female rats were hemiovariectomized and the oviducts and ovary of each animal were separated and autotransplanted under the dorsal skin; four days after the operation, grafted organs and those left *in situ* were collected and GABA content and GAD activity were measured. This procedure was preferred to the neuro-vascular bundle resection, because it allows an easy revascularization of the grafts and consequently leads to minor organ damage. The histological analysis of the oviduct four days after the transplantation, indicated that the organ appeared well preserved and revascularized by capillaries coming from the underlying muscles. Moreover, the tubal cavity was preserved and lined by an epithelium which was, however, much flattened in comparison with that present in diestrous control animals (Celotti *et al.*, 1986). GABA levels and the GAD activity (measured as CO_2 evolution) in the oviduct and ovary after subcutaneous transplantation are shown in table 4; the data are compared to those of the organs left *in situ*. As it can be seen, the grafted tubes possess a much lower GABA content and GAD activity than those present in the *in situ* organs. On the contrary, in the ovary none of the two markers is significantly altered by the autotransplantation. These results were interpreted, at that time, to indicate that the disconnection of the oviduct from the nerve supply was responsible for the decreased activity of the GABAergic system.

Similar results were obtained by other authors in denervation experiments. In all these studies the complete neurovascular bundle was severed, since the selective interruption of the tubal nerve supply is technically impossible. Fernández and coworkers (1985) measured GABA levels in the oviduct after ligation of the ovarian artery bundle. They found a reduction of the GABA content that becomes significant 50 days after surgery and is progressively increased if the observation time is extended to 90 days. The decreased GABA levels do not seem to be caused by generalized changes in the aminoacid composition of the organ since other molecules such as phenylalanine or taurine were not modified by the operation. Moreover, Murashima and Kato (1986) measured the GABA content and the GAD activity in rat oviducts after cutting the ovarian nerve vascular bundle at the ovarian hilus or at the utero-tubal junction. They observed a 50% reduction of both parameters on the operated versus the contralateral organ left intact. However, a reduction of total protein content and creatine kinase activity, although of minor degree when compared to the decrease of GABA and GAD, were present on the operated side and the oviducts were described as atrophic and with granulomatous tissue.

TABLE 4

GABA levels and GAD activity in the oviducts and ovary after subcutaneous grafting

	GABA (nmol/mg prot)		GAD ($nmolCO_2$/ mg prot/hr)	
	control	grafted	control	grafted
Oviduct	55.5±6.1	2.0±0.5*	23.8±2.6	6.6±1.4*
Ovary	1.2±0.2	1.2±0.3	2.6±0.1	2.4±0.1

Values are means ± SEM of at least 5 determinations.
* $p < 0.01$: Student's test.

The transplantation and denervation experiments above described, have led to hypothesize the existence of an extrinsic GABAergic innervation of the oviduct. However, a more cautious interpretation of these data is needed, particularly in the light of some recent biochemical or immunohistochemical observations. GABA-like immunoreactivity (Orensanz *et al.*, 1986) and the GABA-synthesizing machinery (Erdö, 1986a; Murashima and Kato, 1986, Celotti *et al.*, 1989) are restricted to the mucosa of the oviduct. As a matter of fact, the mucosa is probably the tissue most affected by the alteration in blood supply produced by all the denervation procedures.

The first report on the localization of the GABAergic machinery in the oviductal

mucosa came from Murashima and Kato (1986). These authors, utilizing an enzymatic microassay on thick freeze-dry sections of the oviducts and the ovaries, described the presence of much higher levels of GABA and GAD in the mucosa layer of the oviduct than in the muscle. They also showed that the concentration of both GABA and GAD are higher in the isthmic side of the oviduct than in the ampullary one.

More recently, the development of specific antisera raised against GABA, GAD and GABA-T, allowed to confirm also immunohistochemically that the localization of the GABAergic system is restricted to the mucosa of the oviduct. It was demonstrated that GABA immunoreactivity can be detected almost exclusively in the tubal epithelium (Orensanz *et al.*, 1986) being particularly concentrated in the tubular elements of the basal bodies (kinetosomes), with only moderate staining of the cilia (Erdö *et al.*, 1986a). The same authors (Erdö *et al.*, 1986a) found, with an enzyme-histochemical technique, that also GABA-T reactivity was primarily confined in the oviductal epithelium.

The immunolocalization of GAD in the rat oviduct was investigated in our laboratory utilizing both an antibody raised in sheep against rat brain neuronal GAD (Oërtel *et al.*, 1981) and a human autoantibody present in a patient affected by the stiff-man syndrome, a rare disorder characterized by progressive muscle rigidity and endocrine disturbances (Solimena *et al.*, 1988). This autoantibody was shown to cross-react with the rat brain GAD (Solimena *et al.*, 1988).

The immunohistochemical techniques utilized were described elsewhere (Solimena *et al.*, 1988). Briefly, tissues from adult female rats were excised after fixation by transcardiac perfusion; the oviducts were post-fixed and cut at 10 μm with a cryostat. Semi-thin sections (1 μm) were prepared after including tissues in epon. The sections were then incubated with the appropriate antibody and the reaction was visualized by an immunoperoxidase staining. A fully comparable staining was obtained with the sheep antibody and with the patient's serum. In the following figures only sections stained with the patient's autoantibody are presented.

Fig. 1 shows the immunoperoxidase staining of different portions of a rat oviduct. It is apparent that only the mucosa is immuno-stained, while the muscular layer is completely unreactive. The figure also shows that the mucosa of the isthmic side of the oviduct (upper left and lower right) reacts more intensely than that of the ampullary side (upper right).

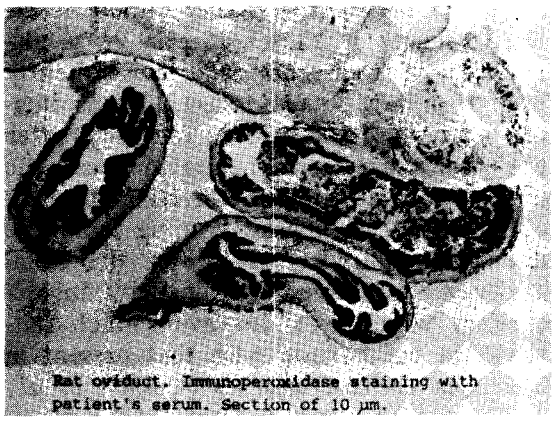

Fig. 1: Immunoperoxidase staining of different portions of the rat oviduct with a human natural glutamic acid decarboxylase autoantibody cross-reacting with the rat enzyme. Section of 10 μm.

Fig. 2 shows a comparison at higher magnification between the ampullary (panel a) and isthmic mucosa (panel b). The staining is more intense in the isthmus, involving mostly the apical part of all cells. In the ampulla it is less intense and seems not to be present in all cells.

The immunohistochemistry on semithin sections (1 μ), performed to achieve a better resolution, indicates that GAD immunoreactivity, both in the ampulla and in the isthmus, is localized in the apical microvilli of the mucosal cells.

Fig. 3 shows that within the isthmus GAD immunoreactivity is localized in the very long stereocilia which are characteristically present in this animal species (Nilsson and Reinius, 1969). This anatomical feature probably explains the more intense staining present in the isthmic portion of the organ.

Fig. 2: High magnification immunoperoxidase staining of the mucosa of two portions (a=ampulla; b=isthmus) of the rat oviduct with a human natural glutamic acid decarboxylase autoantibody cross-reacting with the rat enzyme. Section of 10 μm.

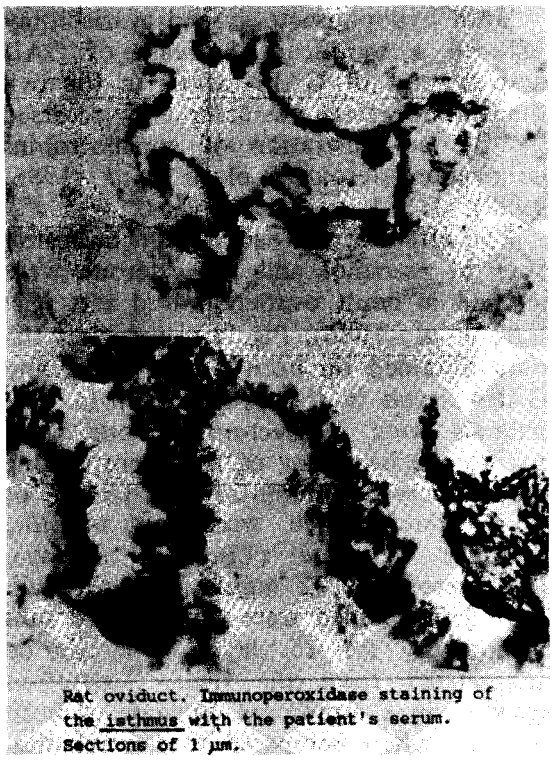

Fig. 3: Immunoperoxidase staining of the isthmic portion of the rat oviduct with a human natural glutamic acid decarboxylase autoantibody cross-reacting with the rat enzyme. Semithin section of 1 μm.

Our results are in partial agreement with recent studies (Erdö *et al.*, 1989). By using a polyclonal anti brain GAD antiserum, it was found specific immunoreaction in the inner layer of the tubal mucosa; however, the immunoreactivity was higher in the ampullary part of the organ than in the isthmic or fimbrial segment. This last evidence is not in agreement with the previous observations by Murashima and Kato (1986) showing the presence of higher GAD activity in the isthmic than in the ampullary segment of the organ.

From the results described above, it appears rather clearly that the GABAergic machinery is localized in the mucosa of the oviduct and not in the muscular layer or in nerve terminals. This supports the hypothesis that the physiological role of GABA in the oviducts might be linked either to the kinetocilia movements or to some paracrine effects that the amino-acid could exert once secreted in the tubal fluid.

Since it is well known that the oviduct is a target for ovarian steroids, we investigated whether the oviductal GABAergic system of the rat could be influenced by physiological (estrous cycle, pregnancy) or experimental (ovariectomy, hypophysectomy, estrogen and/or progesterone replacement) modifications of the sex steroid milieu. As far as the estrous cycle is concerned, slight variations in the GABA content seem to occur along the cycle (Louzan *et al.*, 1986a; Gimeno *et al.*, 1986; Murashima and Kato, 1986; Celotti *et al.*, 1987). Also GAD activity during the estrous cycle does not appear to show any appreciable functuation (Celotti *et al.*, 1987). However, Murashima and Kato (1986) have described a consistent increase of this enzymatic activity in the day of estrous when GAD was assayed in the isolated mucosa of the isthmus.

Both hypophysectomy and ovariectomy induced a significant decrease of GAD activity and of GABA levels in the oviducts. This effect was completely reversed by the administration of gonadotropins or ovarian steroids (Celotti *et al.*, 1986, 1987).

The effect of the exogenous administration of estradiol and progesterone were studied in adult ovariectomized rats (Celotti *et al.*, 1986). Fig. 4 summarizes the data obtained on both GAD activity and GABA levels in the oviduct *in toto*. The animals were ovariectomized one week before the beginning of treatment, taking special care to minimize the damage of the oviduct which were left *in situ*; estradiol benzoate (EB, 50 mg/day), progesterone (P, 2 mg/day) or the two steroids combined were administered subcutaneously for 15 days. As it can be appreciated from the figure, castration resulted in a significant decrease of both markers when compared to normal diestrous animals: the administration of either EB or P alone induced in increase in GAD activity; this is brought back nearly to the normal rat values by the co-administration of the two steroids. On the contrary, steroid treatments (either given separately or in combination) fail to significantly modify GABA concentration. The specificity of the steroid action on the GAD resynthesis is demonstrated by the fact that supernatants of oviducts, excised from animals castrated and treated with EB + P (50 mg and 2 mg/day respectively, for 5 days), show a clear-cut decrease in the GAD activity, when incubated with an anti neuronal GAD antiserum (Celotti *et al.*, 1986).

Since the doses of steroids used in the experiments just described were in the pharmacological range, it was decided to analyze the effect of the administration of low increasing doses of estradiol (from 0.001 mg/day to 6.4 mg/day for 5 days) to ovariectomized animals; the uterine weight was chosen as an index of the known trophic action of the steroid. The results obtained are shown in Fig. 5; as expected, a significant decrease of both uterine weight and GAD activity was observed in castrated animals. Treatment with increasing doses of EB (up to 0.8 mg) produced a progressive growth of the uterus, which, with the higher doses, appeared overstimulated. On the contrary, GAD activity was not significantly modified by EB treatment in this dose range. Similar results were obtained in ovariectomized rats implanted with silastic capsules filled with EB and able to provide a steady state estradiol plasma level of 20-30 pg/ml (see Celotti *et al.*, 1987 for details). From these data, it appears that GABA content and GAD activity are relatively insensitive to ovarian hormones. It might be proposed that, due to the close anatomical connection and to possible direct vascular links with

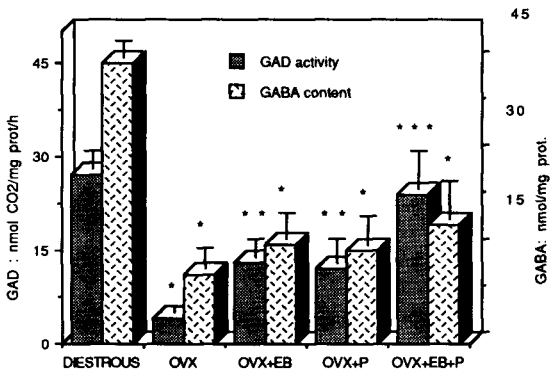

Fig. 4: Effect of estradiol benzoate (EB) and progesterone (P) on glutamic acid decarboxylase (GAD) activity and γ-amino butyric acid (GABA) levels in the oviducts of ovariectomized (ovx) rats. Values represent the mean ± SEM.
* $p < 0.01$ vs. normal diestrous rats; ** $p < 0.05$ vs ovx rats; *** $p < 0.01$ vs ovx rats.

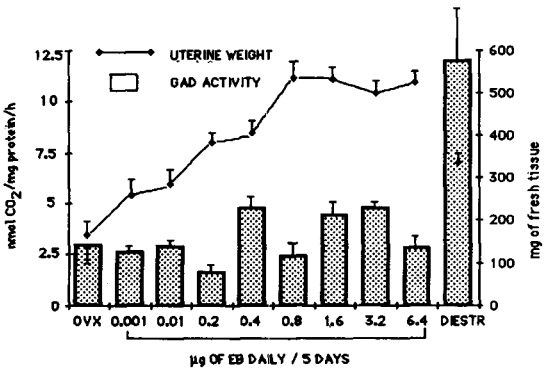

Fig. 5: GAD activity in the oviducts and uterine weight of normal diestrous, OVX and OVX EB-treated rats. Values represent the mean ± SEM. See fig. 4 for abreviations.

the ovary, the oviduct is locally exposed to high levels of ovarian steroids which are difficult to obtain through a subcutaneous administration. Alternatively, it might be postulated that castration induces a partial damage to the tubal blood supply leading to a reduced responsiveness of the mucosa. However, based on the above mentioned findings of Murashima and Kato (1986), it might be hypothesized that changes of the tubal GABAergic system induced by physiological variations of the ovarian steroids, could be revealed only when the mucosa layer is considered.

A different mechanism of regulation of the GABAergic machinery is probably operative during pregnancy. In this physiological situation, in spite of the consistent increase of estrogen secretion, the oviductal GABA content is significantly and persistently decreased (Erdö, 1984; Gimeno *et al.*, 1986).

The physiological role of the very high GABA levels present in the oviduct remains to be determined; the relevant data available so far are shown in Table 5. Since GABA and the enzymes involved in its synthesis and metabolism are localized in the mucosa of the oviduct the most likely role of GABA is in controlling motility of the stereocilia (GABA immunoreactivity is present in basal bodies of the cilia).

The amino acid could be secreted to exert paracrine effects on the adjacent structures such as ovary or uterus. For example high GABA levels, of probable oviductal origin, are present in ovarian bursa fluid and fluctuate during the ovarian cycle (Louzant *et al.*, 1986a). Therefore it is possible that GABA may affect the ovary, modulating either the blood flow or the process of steroidogenesis (Erdö *et al.*, 1985) or may affect the fertilizing capacity of spermatozoa since GABA-A receptors are present in sperm cells (Erdö and Wekerle, 1990).

Cholinergic system

The parasympathetic supply to the oviduct originates from spinal segments S2-S4 in the human (Jansen, 1984) or from L6-S1 in the rat (Pascual *et al.*, 1989); preganglionic fibers distribute through the pelvic splanchnic branches of the corresponding spinal nerves and terminate in gnaglia close to the oviductal isthmus. The postganglionic cholinergic innervation

TABLE 5

Revelant data on the possible physiological role of GABA in the female genital tract

- GABA stimulates spontaneous motility of isolated rabbit oviducts through a GABA-B receptor mechanism (Erdo *et al.*, 1984b).
- GABA does not stimulate spontaneous motility of isolated rat oviducts but potentiate acetylcholine evoked contractions through a GABA-A receptor mechanism (Fernández *et al.*, 1984).
- GABA does not affect leucine incorporation in a ribosomal system isolated from the rat oviducts (Orensanz *et al.*, 1985).
- GABA can be released by preloaded slices of rabbit oviduct under the effect of depolarizing stimuli (Erdö *et al.*, 1986b).
- High GABA levels, possibly of oviductal origin, are found in the fluid of the ovarian bursa (Louzan *et al.*, 1986b).
- GABA superfused on the surface of the ovary increases ovarian blood flow, enhances E2 release and decreases P secretion (Erdö *et al.*, 1986). However removal of oviducts in rats (Celotti *et al.*, unpublished observations) or tubal occlusion in humans (Rivera *et al.*, 1989; Toppozada *et al.*, 1989) do not appear to affect normal cyclicity.
- GABA stimulates spontaneous motility of isolated rabbit uteri through a GABA-B receptor mechanism (Riesz and Erdö, 1985).
- GABA-A receptors are present on spermatozoa (Erdö and Wekerle, 1990).

originates from the ganglia. The ampullary part of the oviduct receives also a parasympathetic supply from the terminal branches of the vagus nerve. Cholinergic fibres appear to be mainly associated with blood vessels.

The data concerning the possible role of acetylcholine on muscle contractility in the female genital tract are relatively scanty. The neurotransmitter added *in vitro* is able to increase the motor activity of circular muscle preparation of human (Owman *et al.*, 1976) and rabbit (Rajkumar and Sharma, 1981) myosalpinx. Moreover, bethanecol, a cholinergic agonist, induces an increase of perfusion resistance in the rabbit oviduct which is antagonized by atropine (Howe, 1976). Since the same author (Howe, 1976) described an analogous antagonistic effect exerted by reserpine and phenoxybenzamine (an alpha-blocking agent) but not by propranolol (a non selective beta-antagonist), it appears that bethanecol might act indirectly via the adrenergic postganglionic release of norepinephrine.

A possible interaction between cholinergic and GABAergic system has been proposed by Fernández and coworkers (1984), who showed that GABA, although inactive on tubal motility by itself, is able to potentiate acetylcholine induced contraction. This effect of GABA is apparently antagonized by bicuculline and therefore possibly mediated via a GABA-A receptor.

On the other hand the sex steroid-induced rise of GAD levels observed in castrated rats can be prevented by atropine and potentiated by neostigmine, an acetylcholinesterase inhibitor, when these drugs are given *in vivo* to castrated rats (Apud, unpublished observations).

The effect of sex steroids on GAD activity in castrated animals was not prevented by treatment either with labetolol (an adrenergic blocking agent) or with alpha metil-tyrosine (a competitive inhibitor of tyrosine hydroxylase), thus indicating that cathecolamines are not involved in sex steroid-induced GAD increase.

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