Chapter II

Background information

Peripheral GABAergic system.

Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the vertebrate central nervous system. Several lines of evidence suggest that GABA may also be a neurotransmitter in the enteric nervous system of mammals (Jessen et al., 1979; Krantis et al., 1980; Jessen and Mirsky, 1982; Taniyama et al., 1982 a.,b.). Thus, the possible neurotransmitter role of GABA has been given increasing attention in the peripheral organs such as intestine (Taniyama et al., 1983 b.; Ong and Kerr, 1984; Hashimoto, Tanaka, and Taniyama, 1986), vas deferens (Bowery et al., 1981), urinary bladder (Kusunoki, Taniyama, and Tanaka, 1984), lung (Erdo and Amenta, 1986) and trachea (Tamaoki, Graf, and Nadel, 1987) have been observed.

Jessen et al.(1979), have shown that GABA is present not only in the central nervous system (CNS), but also in the vertebrate peripheral autonomic nervous system. These investigators have demonstrated the presence, synthesis and uptake of GABA by the myenteric plexus in guinea-pig. The presence of glutamic acid decarboxylase (GAD), the enzyme responsible for conversion of glutamic acid to GABA has also been reported.

The occurrence of GABA and L-glutamate decarboxylase (EC 4.1.1.15), the enzyme primarily responsible for GABA formation in the brain and in peripheral tissues other than gut has been reported (Erdo, Kiss, and

Szporny, 1984; Amenta et al., 1986; Erdo and Amenta, 1986; Erdo et al, 1989). Particularly high GABA levels have been found in the pancreatic islets (Okada, Taniguchi, and Shimida, 1976.) and the fallopian tube (del Rio, 1981; Erdo, Rosdy, and Szporny, 1982) of the rat. Furthermore, specific GABA receptor mediated contractile responses of various smooth muscle tissues such as the gut (Krantis et al., 1980; Ong and Kerr, 1983; Taniyama et al., 1983; Ong and Kerr, 1984; Tonini et al., 1989), the vas deferens (Bowery et al., 1981), the urinary bladder (Taniyama et al., 1983; Shirakawa et al, 1988) certain blood vessels (Suzuki et al., 1984) and the fallopian tube (Erdo et al., 1983; Erdo and Laszlo, 1984; Erdo and Amenta, 1986; Erdo and Maksay, 1988) has been observed.

Among the peripheral tissue examined so far, the rat oviduct has been found to be the richest in GABA (Erdo, Rosdy, and Szporny, 1982). In addition to the high GABA concentration over twice that in whole brain, the presence of the enzymes of GABA metabolism; e.g. glutamate decarboxylase and GABA-2- oxoglutarate aminotransferase in the rat oviduct has also been demonstrated (Del Rio, 1981; Erdo et al., 1984; Amenta et al., 1986). Moreover, specific GABA receptor binding sites have been identified on membranes of the mammalian oviduct (Erdo and Lapis, 1982 a.; Erdo et al., 1983; Erdo and Amenta, 1986; Erdo and Maksay 1988). Specific GABA-receptor mediated contractile responses of isolated rabbit oviduct have been demonstrated (Erdo et al, 1984), and the modulation of rat oviduct contractility by GABA has been reported (Fernandez et al, 1984). These findings suggested that oviductal GABAergic mechanisms may be involved in the regulation of the motility of this organ.

Measurable of GABA found in the rat oviduct was 2.5 times the amount present in the whole brain (Erdo et al., 1982). The maximum GAD activity found in rat oviduct was one-third of that reported for crude homogenates of mouse brain, but 5 times higher than the activity detected in crude preparations of mouse kidney (Drummond and Phillips, 1974). The occurrence in the rat oviduct of GAD and gamma-aminobutyric acid transferase (GABA-T), the enzymes involved in GABA metabolism, has also been reported by Amenta et al.,(1986). Furthermore, specific GABA receptor binding sites have been identified in membranes of the rat (Erdo and Lapis, 1982 a.) and human (Erdo et al., 1983) oviducts.

The number of binding sites in the human fallopian tube appears to be similar, or even higher, than that of the high-affinity binding sites in the human brain (Van Ness and Olsen, 1979; Erdo et al., 1983), and it exceeds about 30-fold the number of receptors found in the rat oviduct (Erdo and Lapis, 1982 a.). These findings might indicate the presence of GABAergic neurons and a possible functional significance of this amino acid in the female reproductive organ.

Fallopian tube GABAergic innervation

Many lines of evidence indicated that some of the properties of ovarian and oviductal GABAergic systems resemble those of neuronal GABAergic systems. In particular, specific GABA receptor binding sites have been identified in both rat and human organs (Schaeffer and Hsueh, 1982, Erdo and Lapis, 1982 a.,b.; Erdo, 1983 a.; Erdo and Laszlo, 1984). Moreover high-affinity GABA uptake systems have been demonstrated in tissue slices

of the rat ovary (Erdo, 1983 a) and fallopian tube (Erdo, 1983 b). These findings might indicate the presence of GABAergic neurons in the two reproductive organs.

Fernandez et al. (1985), reported that surgical ablation of oviductal extrinsic innervation decreased in GABA levels in the rat oviduct, suggested that some of this GABA is conveyed by anterograde transport along GABAergic component of the extrinsic innervation.

The presence of ^{3}H-GABA binding sites in rat and human oviducts and the GABA modulatory effect on acetylcholine-induced contractions (Fernandez et al., 1984) and a GABA direct effect on rabbit oviduct (Erdo et al., 1984) provide an anatomical and pharmacological substrate leading further support to the possibility of GABAergic extrinsic innervation of the oviduct.

GABA receptors

Two pharmacologically and functionally distinct GABA receptor subtypes have recently been identified in the CNS and in certain peripheral tissues of mammals (Bowery et al., 1983). One of these is sensitive to the agonists GABA and muscimol but not baclofen and can be blocked by bicuculline (GABA-A); the other is responsive to baclofen and GABA but responds very weakly to muscimol and not to bicuculline (GABA-B). No potent and selective blocker of the GABA-B receptors has yet been discovered (Andrews and Johnston, 1979; Gottlieb, 1988).

Many lines of evidence indicated that the presence of specific receptor sites for GABA is not restricted to the CNS but that they are widely distributed in peripheral tissue as well (Jessen et al., 1979; Erdo, 1985; Jessen et al., 1987). The female sex organs of various mammals, for instance (Erdo, 1986 a,b), have been found to contain a significant density of specific binding sites for [^{3}H]-GABA and for the GABA-A receptor agonist [^{3}H]-muscimol (Amenta, 1986). The binding of these ligand to membrane of the ovary, oviduct and uterus (Erdo and Laszlo, 1984) could be displaced by compounds known to act competitively at cerebral GABA-A type receptors . This support the view that GABA-A type receptors are present in the reproductive organs of females.

Erdo et al. (1983), reported that the properties of GABA binding sites in the human fallopian tube resemble those of GABA-A receptor. In 1985, Erdo et al., also demonstrated a remarkable density of high-affinity and specific GABA binding sites in the membranes of the human term placenta. These binding sites showed the properties of a GABA-A receptor.

Most of previous receptor binding studies have identified a bicuculline-sensitive type of GABA receptor (GABA-A) in the fallopian tube of the rat and the human. However, the experiment in the rabbit oviduct by Erdo et al. (1984), indicated the presence of GABA-B receptor in the rabbit oviductal musculature which mediate the contractile response differed from the other species studied.

Physiological responses to GABA of Smooth muscle tissues

The contractility of various segments of the gut (Ong and Kerr, 1983) from different mammals can be modulated by local GABA receptors. GABA elicits contractions *via* GABA-A receptors located on postganglionic cholinergic neurons in the myenteric plexus. On the other hand, GABA-B receptors in the gut mediate relaxation by inhibition of postganglionic cholinergic nerves.

As GABA produces contractions and/or relaxations in the isolated mammalian intestine, this compound may produce contraction by stimulating the excitatory receptors mediating the release of ACh from enteric neurons (Krantis et al., 1980) However, there is no direct evidence that GABA acts on a population of excitatory receptors. While looking for the target cells of GABAergic neurons, Taniyama et al. (1983), found that GABA evoked a release of ACh simultaneously with the contraction of the strip of guinea-pig ileum.

The motility of isolated urinary bladder (Taniyama et al., 1983) can be inhibited by GABA. This bicuculline-sensitive effect is presumably mediated *via* GABA-A receptors on cholinergic nerves. The same phenomenon can be observed also *in situ*.

ACh release can be evoked by GABA from isolated strips of gall bladder (Saito et al., 1984). This effect is likely mediated by GABA-A receptors tocated on postganglionic cholinergic neurons.

In isolated pulmonary artery (Starke and Weitzell, 1980), GABA inhibits the contractile response and the release of NE *via* bicuculline-insensitive (GABA-B) receptors. Also GABA relaxes the basilar artery, but the latter effect is likely mediated by GABA-A sites on vascular element (Wu, 1982).

GABA depresses or facilitates respiratory and sympathetic vasomotor (Bowery et al., 1981) activities through both GABA-A and GABA-B receptors at different sites of the respiratory and cardiovascular reflex pathways.

To determine whether GABA affects the contractile response of airway smooth muscle and, if so, what the mechanism of action is. Tamaoki et al. (1987), studied guinea-pig tracheal rings under isometric conditions *in vitro* found that GABA and related substances had no effect on resting tension but reversibly depressed contractions induced by electrical field stimulation and exogenous ACh. They suggested that GABA decreases the contractile response of airway smooth muscle to cholinergic nerve stimulation by inhibiting the evoked release of ACh *via* GABA-A receptors.

GABA and baclofen have been reported to reduce the twitch response induced by electrical stimulation of mouse and guinea-pig vas deferens (Bowery et al., 1981), this effect being due to an action on prejunctional GABA receptor which reduces the transmitter release from the nerve terminals.

Erdo (1983), reported that GABA stimulates the spontaneous contractility of isolated rabbit oviduct *via* GABA-B receptors which most likely located on smooth muscle cells. Similar responses can be observed in uterine strips. While in rat oviduct, reported by Fernandez et al.(1984) showed that the tissue did not respond to GABA, but the ACh contraction was augmented by GABA and its agonist, muscimol, which being antagonized by bicuculline. The mechanism for GABA enhancement of ACh contractions may not be an increase in ACh release, but rather a postsynaptic modulatory effect mediated through GABA-A receptors.

Thus, these receptor-mediated actions of GABA may be involved, *in situ*, in the local modulation of important living functions such as the regulation of enteric motility, ovum transport and fertility, urination and tissue blood flow.